WEST Search History

DATE: Tuesday, September 09, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=ADJ			
L6	l3 and chaffeensis	68	L6
L5	L3 and canis	96	L5
L4	L3 and cnis	0	L4
L3	l1 and l2	247	L3
L2	(diagnosic or detection or detect? or assay or immunoassay or immunoblot or Elisa or Western)	1566078	L2
L1	Ehrlichia	296	L1

END OF SEARCH HISTORY

(FILE 'HOME' ENTERED AT 15:08:27 ON 09 SEP 2003)

FILE 'BIOSIS, SCISEARCH, VETU, VETB, AGRICOLA' ENTERED AT 15:08:39 ON 09 SEP 2003

L1 3273 S EHRLICHIA
L2 1139244 S (IMMUNOBLOT OR IMMUNOASSAY OR WESTERN OR ELISA OR DECTECT? OR
L3 607 S L1 AND L2
L4 214 S L3 AND CANIS
L5 84 S L4 AND CHAFFEENSIS
L6 138 DUP REM L4 (76 DUPLICATES REMOVED)
L7 60 DUP REM L5 (24 DUPLICATES REMOVED)
L8 85 S L2 AND (CANIS) AND CHAFFEENSIS

=>

L8 ANSWER 1 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB A gene encoding a 23.5-kDa ehrlichial morula membrane protein designated MmpA was cloned by screening an Ehrlichia **canis** expression library with convalescent dog sera, which resulted in three positive clones. Sequence analysis of the insert DNAs from all three clones indicated an open reading frame with a size of 666 bp that encodes MmpA. The structural analysis of MmpA indicated that it is a transmembrane protein with extreme hydrophobicity. Southern blot analysis of the HindIII-digested chromosomal DNA demonstrated the presence of a single copy of the mmpA gene in E. **canis** and Ehrlichia **chaffeensis** but not in the human granulocytic ehrlichiosis agent. The mmpA gene was amplified, cloned, and expressed as a fusion protein. Polyclonal antibodies to the recombinant protein (rMmpA) were raised in rabbits. **Western** blot analysis of E. **canis** and E. **chaffeensis** lysates with the anti-rMmpA serum resulted in the presence of an MmpA band only in E. **canis**, not in E. chaffeenesis. Sera from dogs which were either naturally or experimentally infected with E. **canis** recognized the recombinant protein. Double immunofluorescence confocal microscopy studies demonstrated that MmpA was localized mainly on the morula membrane of E. **canis**. Since the morula membrane is the interface between the ehrlichial growing environment and the host cytoplasm, MmpA may play a role in bacterium-host cell interactions.
AN 2003:233285 BIOSIS
DN PREV200300233285
TI Cloning and characterization of an Ehrlichia **canis** gene encoding a protein localized to the morula membrane.
AU Teng, Ching-Hao; Palaniappan, Raghavan U. M.; Chang, Yung-Fu (1)
CS (1) Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, 14853, USA: yc42@cornell.edu USA
SO Infection and Immunity, (April 2003, 2003) Vol. 71, No. 4, pp. 2218-2225. print.
ISSN: 0019-9567.
DT Article
LA English

L8 ANSWER 2 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB A gene encoding a 23.5 KD ehrlichial morula membrane protein designated as MmpA was cloned by screening an Ehrlichia **canis** expression library with convalescent dog sera, which resulted in three positive clones. Sequence analysis of the insert DNA from all the three clones indicated an overlapping open reading frame with a size of 666 bp that encodes for MmpA. The structural analysis of MmpA indicated that it is a transmembrane protein with extreme hydrophobicity. Southern blot analysis of the Hin-dIII digested chromosomal DNA demonstrated the presence of a single copy of mmpA gene in E. **canis** and E. **chaffeensis** but not in the HGE agent. The mmpA gene was amplified, cloned, and expressed as a fusion protein. Polyclonal antibodies to the recombinant protein (r-MmpA) were raised in rabbits. **Western** blot analysis of E. **canis**, and E. **chaffeensis** lysates with the anti-rMmpA serum resulted the presence of a MmpA band only in E. **canis** but not in E. chaffeenesis. Sera from dogs, which were either naturally or experimentally infected with E. **canis**, recognized the recombinant protein. Double immuno-fluorescence confocal microscopy studies demonstrated that the MmpA was localized mainly on the morula membrane of E. **canis**. Since the morula membrane is the interface between the ehrlichial growing environment and the host cytoplasm, MmpA may play a role in bacterial/host-cell interactions.
AN 2002:596955 BIOSIS
DN PREV200200596955
TI Cloning and characterization of an Ehrlichia **canis** gene encoding a protein localized to the morula membrane.
AU Teng, C. H. (1); Chang, Y. F. (1)

CS (1) College of Veterinary Medicine, Cornell University, Ithaca, NY USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (2002) Vol. 102, pp. 178. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print.
Meeting Info.: 102nd General Meeting of the American Society for Microbiology Salt Lake City, UT, USA May 19-23, 2002 American Society for Microbiology
. ISSN: 1060-2011.
DT Conference
LA English

L8 ANSWER 3 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2002:304475 BIOSIS
DN PREV200200304475
TI Human ehrlichioses.
AU Olano, Juan P.; Walker, David H. (1)
CS (1) University of Texas Medical Branch, 301 University Boulevard, Keiller Building, Galveston, TX, 77555-0609: dhwalker@utmb.edu USA
SO Medical Clinics of North America, (March, 2002) Vol. 86, No. 2, pp. 375-392. print.
ISSN: 0025-7125.
DT General Review
LA English

L8 ANSWER 4 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB Canine monocytic ehrlichiosis, caused by Ehrilichia **canis** is a potentially fatal disease of dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. In the invention described herein, a new immunoreactive E. **canis** surface protein gene of 1170-bp was cloned, which encodes a protein with a predicted molecular mass of 42.6 kilodaltons (P43). The P43 gene was not found in E. **chaffeensis** DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. **chaffeensis** by IFA. The P43 was located on the surface of E. **canis** by immunoelectron microscopy. Forty-two dogs exhibiting signs and/or hematologic abnormalities associated with canine ehrlichiosis were tested by IFA and by **Western immunoblot**. Among the 22 samples that were IFA positive for E. **canis**, 100% reacted with the rP43, 96% with the rP28, and 96% with the rP140. The specificity of the recombinant proteins compared to IFA was 96% for rP28, 88% for P43 and 63% for P140. Results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for the diagnosis of Ehrlichia **canis** infections.
AN 2002:278689 BIOSIS
DN PREV200200278689
TI P43 antigen for the immunodiagnosis of canine ehrlichiosis and uses thereof.
AU Walker, David H. (1); McBride, Jere W.
CS (1) Galveston, TX USA
ASSIGNEE: Research Development Foundation
PI US 6355777 March 12, 2002
SO Official Gazette of the United States Patent and Trademark Office Patents, (Mar. 12, 2002) Vol. 1256, No. 2, pp. No Pagination.
http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133.
DT Patent
LA English

L8 ANSWER 5 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB Human granulocytic ehrlichiosis (HGE) is caused by infection with an obligatory intracellular bacterium, the HGE agent. A sensitive and specific nested PCR method was developed based on the p44 multigene family which consists of approximately 20 homologous genes in the genome. The specificity of the PCR was examined with Ehrlichia **canis**, E.

risticii, E. **chaffeensis**, E. sennetsu, E. equi and Anaplasma marginale DNA as templates. The PCR was group-specific, and amplified only the HGE agent, E. equi and A. marginale, but not other ehrlichial DNA. The detection limit of the PCR was 0.16fg of the HGE agent genomic DNA with human blood leukocyte DNA background, which corresponds to approximately two copies of p44 genes in the **assay** mixture. The blood specimens derived from seven culture-positive HGE patients were all positive by the PCR. Blood specimens derived from 15 healthy donors in non-endemic regions were all negative. A total of 26 acute-phase blood specimens from patients suspected of having HGE were examined by the nested PCR using the p44 primers and using primer pairs of GE3a-GE10r and GE9f-GE2 based on the 16S rRNA gene. Of 26 patients, 16 (61.5%) were PCR positive with the p44 primers and 6 (23.1%) were PCR positive with the 16S rDNA primers. Convalescent sera from the 26 patients were tested by **Western** blot analysis. Seven patients (26.9%) reacted with recombinant P44 protein in **Western** blot analysis and all of them were also PCR positive with p44 primers. The nested PCR with the p44 primers, therefore, appears to be more sensitive for the early detection of the HGE infection than the nested PCR with the 16S rDNA primers or rP44 **Western** blotting.

AN 2002:201537 BIOSIS
DN PREV200200201537
TI A nested PCR with the p44-specific primers for diagnosis of the human granulocytic ehrlichiosis.
AU Lin, Q. (1); Zhi, N. (1); Kim, H. (1); Horowitz, H.; Wormser, G.; Rikihisa, Y. (1)
CS (1) Ohio State University, Columbus, OH USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 319. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001
ISSN: 1060-2011.
DT Conference
LA English

L8 ANSWER 6 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB PCR was used to amplify a 537-bp region of an Ehrlichia ewingii gene encoding a homologue of the 28-kDa major antigenic protein (P28) of Ehrlichia **chaffeensis**. The E. ewingii p28 gene homologue was amplified from DNA extracted from whole blood obtained from four humans and one canine with confirmed cases of infection. Sequencing of the PCR products (505 bp) revealed a partial gene with homology to outer membrane protein genes from Ehrlichia and Cowdria spp.: p30 of Ehrlichia **canis** (ltoreq71.3%), p28 of E. **chaffeensis** (ltoreq68.3%), and map1 of Cowdria ruminantium (67.3%). The peptide sequence of the E. ewingii partial gene product was deduced (168 amino acids) and the antigenicity profile was analyzed, revealing a hydrophilic protein with ltoreq69.1% identity to P28 of E. **chaffeensis**, ltoreq67.3% identity to P30 of E. **canis**, and ltoreq63.1% identity to MAP1 of C. ruminantium. Primers were selected from the E. ewingii p28 sequence and used to develop a species-specific PCR diagnostic **assay**. The p28 PCR **assay** amplified the expected 215-bp product from DNA that was extracted from EDTA-treated blood from each of the confirmed E. ewingii infections that were available. The **assay** did not produce PCR products with DNA extracted from E. **chaffeensis**-, E. **canis**-, or E. phagocytophila-infected samples, confirming the specificity of the p28 **assay** for E. ewingii. The sensitivity of the E. ewingii-specific PCR **assay** was evaluated and determined to detect as few as 38 copies of the p28 gene.

AN 2002:7769 BIOSIS
DN PREV200200007769
TI Identification of a p28 Gene in Ehrlichia ewingii: Evaluation of gene for

use as a target for a species-specific PCR diagnostic **assay**.
AU Gusa, Asiya A.; Buller, Richard S.; Storch, Gregory A.; Huycke, Mark M.; Machado, Linda J.; Slater, Leonard N.; Stockham, Steven L.; Massung, Robert F. (1)
CS (1) Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA, 30333: rfm2@cdc.gov USA
SO Journal of Clinical Microbiology, (November, 2001) Vol. 39, No. 11, pp. 3871-3876. print.
ISSN: 0095-1137.
DT Article
LA English

L8 ANSWER 7 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB Forty-nine dogs from Thailand were evaluated for serologic evidence of exposure or polymerase chain reaction (PCR) evidence of infection with vectorborne pathogens, including Ehrlichia sp. (Ehrlichia **canis**, Ehrlichia **chaffeensis**, Ehrlichia equi, and Ehrlichia risticii), Bartonella vinsonii subsp. berkhoffi (Bvb), spotted fever group (SFG) rickettsiae (Rickettsia rickettsii), Typhus group (TG) rickettsiae (Rickettsia canada, Rickettsia prowazekii, and Rickettsia typhi), and Babesia sp. (Babesia **canis** and Babesia gibsonii). All study dogs had at least 1 of 3 entry criteria: fever, anemia, or thrombocytopenia. By immunofluorescence antibody (IFA) testing, seroreactivity was most prevalent to E **chaffeensis** (74%) and E **canis** (71%) antigens, followed by E equi (58%), Bvb (38%), E risticii (38%), R prowazekii (24%), B **canis** (20%), R rickettsii (12%), R canada (4%), and B gibsonii (4%) antigens. There was 100% concordance between E **canis** IFA and **Western** blot **immunoassay** (WI) for 35 of 35 samples; 2 samples were IFA and WI reactive only to E equi antigens. by PCR amplification, 10 dogs were found to be infected with E **canis**, 5 with Ehrlichia platys, and 3 with B **canis**. Sequencing of PCR products was undertaken to compare Ehrlichia strains from Thailand to strains originating from the United States. Partial DNA sequence analysis confirmed infection with E **canis** and E platys, with identical 16S rRNA sequence alignment to E **canis** (U26740) and to E platys (M83801), as reported in GenBank. Partial E **canis** P28.1 and P28.2 amino acid sequences from Thai dogs were divergent from analogous sequences derived from North American E **canis** (AF082744) strains, suggesting that the Thai dogs were infected with a geographically distinct strain of E **canis** compared to North American strains. The results of this study indicate that dogs in Thailand have substantial exposure to vectorborne diseases and that coinfection with these pathogens may be common.
AN 2001:496748 BIOSIS
DN PREV200100496748
TI Serologic and molecular evidence of coinfection with multiple vector-borne pathogens in dogs from Thailand.
AU Suksawat, Jiraporn; Xuejie, Yu; Hancock, Susan I.; Hegarty, Barbara C.; Nilkumhang, Parnchitt; Breitschwerdt, Edward B. (1)
CS (1) Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC, 27606: ed_breitschwerdt@ncsu.edu USA
SO Journal of Veterinary Internal Medicine, (September October, 2001) Vol. 15, No. 5, pp. 453-462. print.
ISSN: 0891-6640.
DT Article
LA English
SL English

L8 ANSWER 8 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:225705 BIOSIS
DN PREV200100225705
TI Evaluation of the MAP1b **ELISA** for the diagnosis of heartwater in South Africa.

AU De Waal, Daniel T. (1); Matthee, Olivier; Jongejan, Frans
CS (1) Parasitology Division, Onderstepoort Veterinary Institute, Onderstepoort, 0110: theo@moon.ovi.ac.za South Africa
SO Society for Tropical Veterinary Medicine. Annals of the New York Academy of Sciences, (December, 2000) Vol. 916, pp. 622-627. Annals of the New York Academy of Sciences. Tropical veterinary diseases: Control and prevention in the context of the new world order. print.
Publisher: New York Academy of Sciences 2 East 63rd Street, New York, NY, 10021, USA.
Meeting Info.: Fifth Biennial Conference of the Society for Tropical Veterinary Medicine Key West, Florida, USA June 12-16, 1999
ISSN: 0077-8923. ISBN: 1-57331-281-9 (cloth), 1-57331-282-7 (paper).
DT Book; Conference
LA English
SL English

L8 ANSWER 9 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB Ehrlichia **canis** causes a potentially fatal rickettsial disease of dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. We recently reported the cloning of two immunoreactive E. **canis** proteins, P28 and P140, that were applicable for serodiagnosis of the disease. In the present study we cloned a new immunoreactive E. **canis** surface protein gene of 1,170 bp, which encodes a protein with a predicted molecular mass of 42.6 kDa (P43). The P43 gene was not detected in E. **chaffeensis** DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. **chaffeensis** as detected by indirect fluorescent antibody (IFA) **assay**. Forty-two dogs exhibiting signs and/or hematologic abnormalities associated with canine ehrlichiosis were tested by IFA **assay** and by recombinant **Western immunoblot**. Among the 22 samples that were IFA positive for E. **canis**, 100% reacted with rP43, 96% reacted with rP28, and 96% reacted with rP140. The specificity of the recombinant proteins compared to the IFAs was 96% for rP28, 88% for P43 and 63% for P140. The results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for E. **canis** infections.
AN 2001:84427 BIOSIS
DN PREV200100084427
TI Immunodiagnosis of Ehrlichia **canis** infection with recombinant proteins.
AU McBride, Jere W.; Corstvet, Richard E.; Breitschwerdt, Edward B.; Walker, David H. (1)
CS (1) Department of Pathology, University of Texas Medical Branch, 301 University Blvd., Galveston, TX, 77555-0609: dwalker@utmb.edu USA
SO Journal of Clinical Microbiology, (January, 2001) Vol. 39, No. 1, pp. 315-322. print.
ISSN: 0095-1137.
DT Article
LA English
SL English

L8 ANSWER 10 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:524802 BIOSIS
DN PREV200000524802
TI Naturally occurring Ehrlichia **chaffeensis** infection in coyotes from Oklahoma.
AU Kocan, Alan (1); Levesque, Gena Crowder; Whitworth, Lisa C.; Murphy, George L.; Ewing, Sidney A.; Barker, Robert W.
CS (1) Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, 74078 USA
SO Emerging Infectious Diseases, (Oct., 2000) Vol. 6, No. 5, pp. 477-480. print.
ISSN: 1080-6040.

DT Article
LA English
SL English

L8 ANSWER 11 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB Background Human ehrlichiosis is a recently recognized tick-borne infection. Four species infect humans: Ehrlichia **chaffeensis**, E. sennetsu, E. **canis**, and the agent of human granulocytic ehrlichiosis. Methods We tested peripheral-blood leukocytes from 413 patients with possible ehrlichiosis by broad-range and species-specific polymerase-chain-reaction (PCR) **assays** for ehrlichia. The species present were identified by species-specific PCR **assays** and nucleotide sequencing of the gene encoding ehrlichia 16S ribosomal RNA. **Western** blot analysis was used to study serologic responses. Results In four patients, ehrlichia DNA was detected in leukocytes by a broad-range PCR **assay**, but not by **assays** specific for E. **chaffeensis** or the agent of human granulocytic ehrlichiosis. The nucleotide sequences of these PCR products matched that of E. ewingii, an agent previously reported as a cause of granulocytic ehrlichiosis in dogs. These four patients, all from Missouri, presented betweenMay and August 1996, 1997, or 1998 with fever, headache, and thrombocytopenia, with or without leukopenia. All had been exposed to ticks, and three were receiving immunosuppressive therapy. Serum samples obtained from three of these patients during convalescence contained antibodies that reacted with E. **chaffeensis** and E. **canis** antigens in a pattern different from that of humans with E. **chaffeensis** infection but similar to that of a dog experimentally infected with E. ewingii. Morulae were identified in neutrophils from two patients. All four patients were successfully treated with doxycycline. Conclusions These findings provide evidence of E. ewingii infection in humans. The associated disease may be clinically indistinguishable from infection caused by E. **chaffeensis** or the agent of human granulocytic ehrlichiosis.
AN 1999:434994 BIOSIS
DN PREV199900434994
TI Ehrlichia ewingii, a newly recognized agent of human ehrlichiosis.
AU Buller, Richard S.; Arens, Max; Hmiel, S. Paul; Paddock, Christopher D.; Sumner, John W.; Rikihisa, Yasuko; Unver, Ahmet; Gaudreault-Keener, Monique; Manian, Farrin A.; Liddell, Allison M.; Schmulewitz, Nathan; Storch, Gregory A. (1)
CS (1) Department of Pediatrics, Division of Infectious Diseases, St. Louis Children's Hospital, 1 Children's Pl., St. Louis, MO, 63110 USA
SO New England Journal of Medicine, (July 15, 1999) Vol. 341, No. 3, pp. 148-155.
ISSN: 0028-4793.
DT Article
LA English
SL English

L8 ANSWER 12 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:323180 BIOSIS
DN PREV199900323180
TI **Western** and dot blotting analysis of Ehrlichia **chaffeensis**-IFA positive and -negative human sera using native and recombinant E. **chaffeensis** and E. **canis** antigen.
AU Unver, A. (1); Ohashi, N. (1); Rikihisa, Y. (1); Cullman, L. C.
CS (1) Ohio State University, Columbus, OH USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (1999) Vol. 99, pp. 236.
Meeting Info.: 99th General Meeting of the American Society for Microbiology Chicago, Illinois, USA May 30-June 3, 1999 American Society for Microbiology
. ISSN: 1060-2011.
DT Conference

LA English

L8 ANSWER 13 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB Cowdria ruminantium is the etiologic agent of heartwater, a disease causing major economic loss in ruminants in sub-Saharan Africa and the Caribbean. Development of a serodiagnostic test is essential for determining the carrier status of animals from regions where heartwater is endemic, but most available tests give false-positive reactions with sera against related Erhlichia species. Current approaches rely on molecular methods to define proteins and epitopes that may allow specific diagnosis. Two major antigenic proteins (MAPs), MAP1 and MAP2, have been examined for their use as antigens in the serodiagnosis of heartwater. The objectives of this study were (i) to determine if MAP2 is conserved among five geographically divergent strains of C. ruminantium and (ii) to determine if MAP2 homologs are present in Ehrlichia **canis**, the causative agent of canine ehrlichiosis, and Ehrlichia **chaffeensis**, the organism responsible for human monocytic ehrlichiosis. These two agents are closely related to C. ruminantium. The map2 gene from four strains of C. ruminantium was cloned, sequenced, and compared with the previously reported map2 gene from the Crystal Springs strain. Only 10 nucleic acid differences between the strains were identified, and they translate to only 3 amino acid changes, indicating that MAP2 is highly conserved. Genes encoding MAP2 homologs from E. **canis** and E. **chaffeensis** also were cloned and sequenced. Amino acid analysis of MAP2 homologs of E. **chaffeensis** and E. **canis** with MAP2 of C. ruminantium revealed 83.4 and 84.4% identities, respectively. Further analysis of MAP2 and its homologs revealed that the whole protein lacks specificity for heartwater diagnosis. The development of epitope-specific **assays** using this sequence information may produce diagnostic tests suitable for C. ruminantium and also other related rickettsiae.
AN 1999:176106 BIOSIS
DN PREV199900176106
TI Potential value of major antigenic protein 2 for serological diagnosis of heartwater and related Ehrlichial infections.
AU Bowie, Michael V. (1); Reddy, G. Roman; Semu, Shalt M.; Mahan, Suman M.; Barbet, Anthony F.
CS (1) Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32610 USA
SO Clinical and Diagnostic Laboratory Immunology, (March, 1999) Vol. 6, No. 2, pp. 209-215.
ISSN: 1071-412X.
DT Article
LA English

L8 ANSWER 14 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB The major outer membrane proteins (OMPs) of the human granulocytic ehrlichiosis (HGE) agent, with molecular sizes of 44 to 47 kDa, are immunodominant antigens in human infection. Monoclonal antibodies (MAbs) to the OMPs were made by immunizing BALB/c mice with the purified HGE agent and then by fusing spleen cells with myeloma cells. The immunologic specificities of three MAbs (3E65, 5C11, and 5D13) were examined with five human HGE agent isolates and one tick isolate. By **Western** blot analysis, all three MAbs recognized the HGE agent but not Ehrlichia **chaffeensis**, Ehrlichia sennetsu, Ehrlichia **canis**, or their host cells. MAb 3E65 reacted with a 44-kDa protein in the homologous human isolate but not in the remaining five isolates. The two remaining MAbs recognized proteins with molecular sizes of 44 to 47 kDa in all six isolates. **Western** blot results with the OMP fraction of the six isolates were consistent with results with the whole HGE agent. Immunofluorescent-antibody staining and immunogold labeling with these MAbs showed that these antigens were primarily present on the membrane of the HGE agent. MAbs 5C11 and 5D13 recognized the recombinant 44-kDa protein by **Western immunoblot** analysis, but MAb 3E65 did not. Passive immunization with MAb 3E65 was more effective in

protecting mice from HGE agent infection than with MAbs 5C11 and 5D13. These MAbs would be useful for analyzing the role of the major OMP antigens in HGE agent infection and for serodiagnosis.

AN 1998:497705 BIOSIS
DN PREV199800497705
TI Characterization of monoclonal antibodies to the 44-kilodalton major outer membrane protein of the human granulocytic ehrlichiosis agent.
AU Kim, Hyung-Yong; Rikihisa, Yasuko (1)
CS (1) Dep. Vet. Biosci., Coll. Vet. Med., Ohio State Univ., 1925 Coffey Rd., Columbus, OH 43210-1093 USA
SO Journal of Clinical Microbiology, (Nov., 1998) Vol. 36, No. 11, pp. 3278-3284.
ISSN: 0095-1137.
DT Article
LA English

L8 ANSWER 15 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB A 30-kDa major outer membrane protein of Ehrlichia **canis**, the agent of canine ehrlichiosis, is the major antigen recognized by both naturally and experimentally infected dog sera. The protein cross-reacts with a serum against a recombinant 28-kDa protein (rP28), one of the outer membrane proteins of a gene (omp-1) family of Ehrlichia **chaffeensis**. Two DNA fragments of E. **canis** were amplified by PCR with two primer pairs based on the sequences of E. **chaffeensis** omp-1 genes, cloned, and sequenced. Each fragment contained a partial 30-kDa protein gene of E. **canis**. Genomic Southern blot analysis with the partial gene probes revealed the presence of multiple copies of these genes in the E. **canis** genome. Three copies of the entire gene (p30, p30-1, and p30a) were cloned and sequenced from the E. **canis** genomic DNA. The open reading frames of the two copies (p30 and p30-1) were tandemly arranged with an intergenic space. The three copies were similar but not identical and contained a semivariable region and three hypervariable regions in the protein molecules. The following genes homologous to three E. **canis** 30-kDa protein genes and the E. **chaffeensis** omp-1 family were identified in the closely related rickettsiae: wsp from Wolbachia sp., p44 from the agent of human granulocytic ehrlichiosis, msp-2 and msp-4 from Anaplasma marginale, and map-1 from Cowdria ruminantium. Phylogenetic analysis among the three E. **canis** 30-kDa proteins and the major surface proteins of the rickettsiae revealed that these proteins are divided into four clusters and the two E. **canis** 30-kDa proteins are closely related but that the third 30-kDa protein is not. The p30 gene was expressed as a fusion protein, and the antibody to the recombinant protein (rP30) was raised in a mouse. The antibody reacted with rP30 and a 30-kDa protein of purified E. **canis**. Twenty-nine indirect fluorescent antibody (IFA)-positive dog plasma specimens strongly recognized the rP30 of E. **canis**. To evaluate whether the rP30 is a suitable antigen for serodiagnosis of canine ehrlichiosis, the immunoreactions between rP30 and the whole purified E. **canis** antigen were compared in the dot **immunoblot assay**. Dot reactions of both antigens with IFA-positive dog plasma specimens were clearly distinguishable by the naked eye from those with IFA-negative plasma specimens. By densitometry with a total of 42 IFA-positive and -negative plasma specimens, both antigens produced results similar in sensitivity and specificity. These findings suggest that the rP30 antigen provides a simple, consistent, and rapid serodiagnosis for canine ehrlichiosis. Cloning of multigenes encoding the 30-kDa major outer membrane proteins of E. **canis** will greatly facilitate understanding pathogenesis and immunologic study of canine ehrlichosis and provide a useful tool for phylogenetic analysis.

AN 1998:435123 BIOSIS
DN PREV199800435123
TI Cloning and characterization of multigenes encoding the immunodominant 30-kilodalton major outer membrane proteins of Ehrlichia **canis**

and application of the recombinant protein for serodiagnosis.
AU Ohashi, Norio; Unver, Ahmet; Zhi, Ning; Rikihisa, Yasuko (1)
CS (1) Dep. Veterinary Biosciences, Coll. Veterinary Med., Ohio State Univ., 1925 Coffey Rd., Columbus, OH 43210-1093 USA
SO Journal of Clinical Microbiology, (Sept., 1998) Vol. 36, No. 9, pp. 2671-2680.
ISSN: 0095-1137.
DT Article
LA English

L8 ANSWER 16 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB In order to evaluate the relative sensitivity of the detection of antibodies against various antigenic proteins of Ehrlichia **chaffeensis** for the diagnosis of the emerging infectious disease human monocytotropic ehrlichiosis, **Western** immunoblotting was performed with 27 serum samples from convalescent patients with antibodies, as demonstrated by indirect immunofluorescence **assay** . Among 22 patients with antibodies reactive with the 120-kDa protein, 15 showed reactivity with the 29/28-kDa protein(s) and the proteins in the 44- to 88-kDa range. Two of the serum samples with this pattern reacted with the 29/28-kDa protein(s) of only the 91HE17 strain, and one sample reacted with only that of the Arkansas strain, indicating that the antibodies were stimulated by strain-specific epitopes. Overall, antibodies to the 29/28-kDa protein(s) were detected in only 16 patients' sera, suggesting that this protein is less sensitive than the 120-kDa protein. Two of 12 serum samples from healthy blood donors had antibodies reactive with the 120-kDa protein; one of these samples reacted also with the 29/28-kDa protein(s) of Ehrlichia **canis**, suggesting that unrecognized ehrlichial infection might have occurred, including human infection with E. **canis**. A high correlation between reactivity with the 120-kDa protein by **Western** immunoblotting and the recombinant 120-kDa protein by dot blot supports the potential usefulness of this recombinant antigen in diagnostic serology.
AN 1998:33569 BIOSIS
DN PREV199800033569
TI **Western** immunoblotting analysis of the antibody responses of patients with human monocytotropic Ehrlichiosis to different strains of Ehrlichia **chaffeensis** and Ehrlichia **canis**.
AU Chen, Sheng-Min; Cullman, Louis C.; Walker, David H. (1)
CS (1) Dep. Pathol., Univ. Texas Med. Branch, 301 University Blvd., Galveston, TX 77555-0609 USA
SO Clinical and Diagnostic Laboratory Immunology, (Nov., 1997) Vol. 4, No. 6, pp. 731-735.
ISSN: 1071-412X.
DT Article
LA English

L8 ANSWER 17 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB A partial 16S rRNA gene was amplified in Ehrlichia **canis** -infected cells by nested PCR. The **assay** was specific and did not amplify the closely related Ehrlichia **chaffeensis**, Ehrlichia muris, Neorickettsia helminthoeca, and SF agent 16S rRNA genes. The **assay** was as sensitive as Southern hybridization, detecting as little as 0.2 pg of E. **canis** DNA. By this method, all blood samples from four dogs experimentally infected with E. **canis** were positive as early as day 4 postinoculation, which was before or at the time of seroconversion. One hundred five blood samples from dogs from Arizona and Texas (areas of E. **canis** endemicity) and 30 blood samples from dogs from Ohio (area of E. **canis** nonendemicity) were examined by nested PCR and immunofluorescent-antibody (IFA) test. Approximately 84% of dogs from Arizona and Texas bad been treated with doxycycline before submission of blood specimens. Among Arizona and Texas specimens, 46 samples were PCR positive (44%) and 80 were IFA positive (76%). Forty-three of 80 IFA-positive samples (54%) were PCR positive, and

22 of 25 IFA-negative samples (88%) were negative in the nested PCR. None of the Ohio specimens were IFA positive, but 5 specimens were PCR positive (17%). Our results indicate that the nested PCR is highly sensitive and specific for detection of E. **canis** and may be more useful in assessing the clearance of the organisms after antibiotic therapy than IFA, especially in areas in which E. **canis** is endemic.

AN 1997:304783 BIOSIS
DN PREV199799612586
TI Comparison of nested PCR with immunofluorescent-antibody **assay** for detection of Ehrlichia **canis** infection in dogs treated with doxycycline.
AU Wen, Bohai; Rikihisa, Yasuko (1); Mott, Jason M.; Greene, Russell; Kim, Hyung-Yong; Zhi, Ning; Couto, Guillermo C.; Unver, Ahmet; Bartsch, Robert
CS (1) Dep. Veterinary Biosciences, Coll. Veterinary Med., Ohio State Univ., 1925 Coffey Rd., Columbus, OH 43210-1096 USA
SO Journal of Clinical Microbiology, (1997) Vol. 35, No. 7, pp. 1852-1855. ISSN: 0095-1137.
DT Article
LA English

L8 ANSWER 18 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB Recombinant baculovirus techniques were used to express the 260 amino acid carboxyterminal portion of the 32 kilodalton (kDa) major antigenic protein (MAP 1) of Cowdria ruminantium, the heartwater agent, as a fusion protein. The recombinant MAP 1 was fused to an aminoterminal independently antigenic octapeptide sequence (FLAG peptide). Recombinant MAP I was used as an immunoblotting antigen to evaluate numerous reference antisera against organisms of the tribe Ehrlichieae. Monoclonal and polyclonal C. ruminantum antibodies, monoclonal anti-FLAG ascites, and antisera to Ehrlichia **canis** and Ehrlichia **chaffeensis** reacted with this antigen. Twelve of 79 sera collected 1980 to 1992 from southeastern U.S. white-tailed deer (Odocoileus virginianus) were also unexpectedly **immunoblot**-positive to MAP 1. These 12 deer sera had, as a group, significantly (P lt 0.01) greater anti-E. **chaffeensis** titers (previously determined) than the sera from MAP 1 **immunoblot** -negative deer living in the same areas. None of the 262 sera from cattle living in the same areas were **immunoblot**-positive to MAP 1. All of an additional 50 cervine sera from Michigan (USA), 72 bovine sera from northern U.S. cattle, and 72 sera from Puerto Rican cattle were also **immunoblot**-negative to MAP 1. Sera from African sheep which were falsely seropositive to authentic MAP 1 were also **immunoblot** -positive to the recombinant MAP 1. Unidentified Ehrlichia spp. capable of serologic crossreactivity with the heartwater agent appear to be present in some southeastern U.S. white-tailed deer but not cattle. These or related Ehrlichia spp. may also be found elsewhere in the world in non-cervine species.

AN 1996:509203 BIOSIS
DN PREV199699231559
TI A recombinant antigen from the heartwater agent (Cowdria ruminatium) reactive with antibodies in some southeastern United States white-tailed deer (Odocoileus virginianus), but not cattle, sera.
AU Katz, Jonathan B. (1); Barbet, Anthony F.; Mahan, Suman M.; Kumbula, David; Lockhart, J. Mitchell; Keel, M. Kevin; Dawson, Jacqueline E.; Olson, James G.; Ewing, Sidney A.
CS (1) Diagn. Virol. Lab., Natl. Vet. Serv. Lab., Vet. Serv., Anim. Plant Health Inspection Serv., U.S. Dep. Agric., Ames, IA 50010 USA
SO Journal of Wildlife Diseases, (1996) Vol. 32, No. 3, pp. 424-430. ISSN: 0090-3558.
DT Article
LA English

L8 ANSWER 19 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB We report the first isolation and molecular and antigenic characterization of a human ehrlichial species in South America. A retrospective study was

performed with serum specimens from 6 children with clinical signs suggestive of human ehrlichiosis and 43 apparently healthy adults who had a close contact with dogs exhibiting clinical signs compatible with canine ehrlichiosis. The evaluation was performed by the indirect fluorescent-antibody **assay** with Ehrlichia **chaffeensis** Arkansas, Ehrlichia **canis** Oklahoma, and Ehrlichia muris antigens. The sera from two apparently healthy humans were positive by the indirect fluorescent-antibody **assay** for all three antigens. Of the three antigens, samples from humans 1 and 2 showed the highest antibody titers against E. **chaffeensis** and E. muris, respectively. The remaining serum samples were negative for all three antigens. One year later examination of a blood sample from subject 1 revealed morulae morphologically resembling either E. **canis**, E. **chaffeensis**, or E. muris in monocytes in the blood smear. The microorganism, referred to here as Venezuelan human ehrlichia (VHE), was isolated from the blood of this person at 4 days after coculturing isolated blood leukocytes with a dog macrophage cell line (DH82). The organism was also isolated from mice 10 days after intraperitoneal inoculation of blood leukocytes from subject 1. Analysis by electron microscopy showed that the human isolate was ultrastructurally similar to E. **canis**, E. **chaffeensis**, and E. muris. When the virulence of VHE in mice was compared with those of E. **chaffeensis** , E. **canis**, and E. muris, only VHE and E. muris induced clinical signs in BALB/c mice at 4 and 10 days, respectively, after intraperitoneal inoculation. VHE was reisolated from peritoneal exudate cells of the mice. Only E. **chaffeensis**- and E. muris-infected mice developed significant splenomegaly. **Western immunoblot** analysis showed that serum from subject 1 reacted with major proteins of the VHE antigen of 110, 80, 76, 58, 43, 35, and 34 kDa. Human serum against E. **chaffeensis** reacted strongly with 58-, 54-, 52-, and 40-kDa proteins of the VHE antigen. Anti-E. **canis** dog serum reacted strongly with 26- and 24-kDa proteins of VHE. In contrast, anti-E. sennetsu rabbit and anti-E. muris mouse sera did not react with the VHE antigen. Serum from subject I reacted with major proteins of 90, 64, or 47 kDa of the E. **chaffeensis**, E. **canis**, and E. muris antigens. This reaction pattern suggests that this serum sample was similar to serum samples from E. **chaffeensis**-infected human patients in Oklahoma. The base sequence of the 16S rRNA gene of VHE was most closely related to that of E. **canis** Oklahoma. On the basis of these observations, we suggest that VHE is a new strain or a subspecies of E. **canis** which may cause asymptomatic persistent infection in humans.

AN 1996:464006 BIOSIS
DN PREV199699186362
TI Ehrlichia **canis**-like agent isolated from a man in Venezuela: Antigenic and genetic characterization.
AU Perez, Miriam; Rikihisa, Yasuko (1); Wen, Bohai
CS (1) Dep. Vet. Biosci., Coll. Vet. Med., Ohio State Univ., Columbus, OH 43210 USA
SO Journal of Clinical Microbiology, (1996) Vol. 34, No. 9, pp. 2133-2139. ISSN: 0095-1137.
DT Article
LA English

L8 ANSWER 20 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB Objective: To ascertain whether dogs are naturally infected with Ehrlichia **chaffeensis**. Animals: 74 dogs from 5 animal shelters and 1 kennel in 3 cities and 3 counties in southeastern Virginia were tested during June 1991. Procedure: Blood was drawn from 74 dogs; 73 were tested serologically for antibodies reactive to E. **chaffeensis** and E. **canis**, and 38 were tested for the presence of E. **chaffeensis**, E. **canis**, and E. ewingii by polymerase chain reaction (PCR). Serologic testing by indirect fluorescent antibody **assay**. Nested PCR used Ehrlichia-wide outside primers to detect

initial products, followed by use of species-specific primers for identification. Results: 28 (38.4%) dogs had a positive test result (minimum titer, gtoreq 1:64) for antibodies reactive to E. **chaffeensis**, and 28 (38.4%) had a positive reaction to E. **canis**. PCR analysis indicated that 8 (42.1%) dogs were positive for E. **chaffeensis** and 6 dogs (31.6%) were positive for E. ewingii. All dogs had negative results of the PCR test for E. **canis**. Conclusion: Dogs are potential reservoirs of E. **chaffeensis**. Clinical Relevance: Canine E. **chaffeensis** infection may be more prevalent than E. **canis** or E. ewingii infection in this region of the United States.

AN 1996:422597 BIOSIS
DN PREV199699153653
TI Polymerase chain reaction evidence of Ehrlichia **chaffeensis**, an etiologic agent of human ehrlichiosis, in dogs from southeast Virginia.
AU Dawson, Jacqueline E. (1); Biggie, Kristine L. (1); Warner, Cynthia K. (1); Cookson, Kalen; Jenkins, Suzanne; Levine, Jay F.; Olson, James G. (1)
CS (1) Viral Rickettsial Zoonoses Branch, Div. Viral Rickettsial Dis., Natl. Cent. Infect. Dis., CDC, Atlanta, Ga 30333 USA
SO American Journal of Veterinary Research, (1996) Vol. 57, No. 8, pp. 1175-1179.
ISSN: 0002-9645.
DT Article
LA English

L8 ANSWER 21 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB Ehrlichia **chaffeensis**, an obligately intracellular bacterium with tropism for monocytes, is the etiologic agent of human monocytic ehrlichiosis. To determine the nature and ultrastructural location of E. **chaffeensis** antigens, monoclonal antibodies (MAbs) to E. **chaffeensis** were developed. The MAbs were used for immunofluorescence and **Western** immunoblotting analysis of the antigens of density gradient-purified ehrlichiae. Monoclonal antibody 6AI recognized an epitope of a 30-kD protein. This antibody reacted with a strain-specific epitope of E. **chaffeensis**, Arkansas strain, and did not cross-react with any other ehrlichia tested. Monoclonal antibodies 3C7 and 7C1-B recognized Arkansas strain proteins of 30 and 29 kD and reacted with E. **chaffeensis** (strain 91HE17) proteins of 31 and 29 kD and an E. **canis** protein of 30 kD. Lack of reactivity of these two MAbs with E. sennetsu and E. risticii suggests that the epitope is group-specific. Monoclonal antibody 5D11 recognized a 58-kD protein of both strains of E. **chaffeensis** as well as E. **canis**, apparently a group-specific, conformation-independent epitope. Monoclonal antibody 7C1-C reacted with 58- and 88-kD proteins of both the Arkansas and 91HE17 strains. Trypsin treatment destroyed the reactivity of E. **chaffeensis** antigens with all the MAbs when tested by **Western** immunoblotting, indicating that these antigens are proteins with trypsin-sensitive epitopes. Immunoelectron microscopy of negatively stained intact E. **chaffeensis** organisms showed that the 30- and 29-kD proteins are present on the surface of the ehrlichial cell wall along with the previously localized 28-kD protein.

AN 1996:285097 BIOSIS
DN PREV199699007453
TI Analysis and ultrastructural localization of Ehrlichia **chaffeensis** proteins with monoclonal antibodies.
AU Chen, Sheng-Min; Popov, Vsevolod L.; Feng, Hui-Min; Walker, David H.
CS Dep. Pathol., Univ. Tex. Med. Branch, Galveston, TX 77555-0609 USA
SO American Journal of Tropical Medicine and Hygiene, (1996) Vol. 54, No. 4, pp. 405-412.
ISSN: 0002-9637.
DT Article
LA English

L8 ANSWER 22 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AB Currently available serological tests for cowdriosis (Cowdria ruminantium infection) in domestic ruminants are hampered by their low specificities because of cross-reactivity with Ehrlichia spp. The use of recombinant major antigenic protein (MAP1) of C. ruminantium for serodiagnosis was investigated. Overlapping fragments of the MAP1 protein were expressed in Escherichia coli and were reacted with sera from sheep infected with either C. ruminantium or Ehrlichia ovina. Two immunogenic regions on the MAP1 protein, designated MAP1-A and MAP1-B, were identified. MAP1-A was reactive with C. ruminantium antisera, E. ovina antisera, and three MAP1-specific monoclonal antibodies, whereas MAP1-B reacted only with C. ruminantium antisera. An indirect enzyme-linked immunosorbent **assay** (**ELISA**) based on MAP1-B was further developed and validated with sera from animals experimentally infected with C. ruminantium or several Ehrlichia spp. Antibodies raised in sheep, cattle, and goats against nine isolates of C. ruminantium reacted with MAP1-B. Cross-reactivity with MAP1-B was limited to Ehrlichia **canis** and Ehrlichia **chaffeensis**, two rickettsias which do not infect ruminants. Antibodies to Ehrlichia spp. which do infect ruminants (E. bovis, E. ovina, and E. phagocytophila) did not react with MAP1-B. Antibody titers to C. ruminantium in sera from experimentally infected cattle, goats, and sheep were detectable for 50 to 200 days postinfection. Further validation of the recombinant MAP1-B-based **ELISA** was done with sera obtained from sheep raised in heartwater-free areas in Zimbabwe and from several Caribbean islands. A total of 159 of 169 samples which were considered to be false positive by immunoblotting or indirect **ELISA** did not react with MAP1-B. In conclusion, recombinant MAP1-B may be a suitable antigen for a sensitive serological test for cowdriosis, with dramatically improved specificity.
AN 1995:439402 BIOSIS
DN PREV199598453702
TI Use of a specific immunogenic region on the Cowdria ruminantium MAP1 protein in a serological **assay**.
AU Van Vliet, Arnoud H. M.; Van Der Zeijst, Bernard A. M.; Camus, Emmanuel; Mahan, Suman M.; Martinez, Dominique; Jongejan, Frans (1)
CS (1) Inst. Infectious Diseases Immunol., P.O. Box 80 165, 3508 TD Utrecht Netherlands
SO Journal of Clinical Microbiology, (1995) Vol. 33, No. 9, pp. 2405-2410. ISSN: 0095-1137.
DT Article
LA English

L8 ANSWER 23 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB Human monocytic ehrlichiosis is caused by Ehrlichia **chaffeensis**, an intracellular bacterium probably transmitted by the tick Amblyomma americanum in the United States. Despite its lack of specificity in discriminating among infections by closely related Ehrlichia spp., immunofluorescence **assay** (IFA) is the most frequently used serological diagnostic method. To improve the specificity of the serological diagnosis, we compared antigenic profile of E. **canis** and E. **chaffeensis** antigen with homologous and heterologous sera, searching for the specificity of the presence of low-molecular-weight proteins. **Western immunoblot** analysis of IFA-positive human sera revealed 27- and 29-kDa proteins which are not found in E. **canis** IFA-positive sera from dogs. IFA-positive sera from dogs revealed a low-molecular-weight group of proteins (20 to 28 kDa) which were not found in human E. **chaffeensis**-positive sera except for a weak band at 22 kDa. The presence of antibodies directed against the 27- and 29-kDa proteins on **Western** blots is specific for E. **chaffeensis** infection, and we suggest that the **Western** blot might complete IFA in cases with low positive predictive value.
AN 1995:109956 BIOSIS
DN PREV199598124256
TI Serologic diagnosis of human monocytic ehrlichiosis by **immunoblot**

analysis.
AU Brouqui, P. (1); Lecam, C.; Olson, J.; Raoult, D.
CS (1) Unite de Rickettsies, Faculte de Medecine, 27 Blvd. J. Moulin, 13385 Marseilles Cedex 5 France
SO Clinical and Diagnostic Laboratory Immunology, (1994) Vol. 1, No. 6, pp. 645-649.
ISSN: 1071-412X.
DT Article
LA English

L8 ANSWER 24 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB Ehrlichia **chaffeensis**, E. **canis**, and E. ewingii are genetically closely related, as determined by 16S rRNA gene base sequence comparison, but they exhibit biologic differences. E. **chaffeensis** is the etiologic agent of human ehrlichiosis. E. **canis** and E. ewingii cause two distinctly different forms of canine ehrlichiosis and infect different types of leukocytes, monocytes and granulocytes, respectively. E. **chaffeensis** can also infect dogs. In the study, **Western immunoblot** analysis of sera from dogs inoculated with E. **chaffeensis**, E. **canis**, or E. ewingii was performed to determine antigenic specificity and the intensities of the reactions to purified E. **chaffeensis** and E. **canis** antigens. At 2 to 3 weeks postexposure, antisera from four dogs inoculated with E. **chaffeensis** reacted with 64-, 47-, 31-, and 29-kDa proteins of E. **chaffeensis** but reacted poorly with E. **canis** antigen. In contrast, at 2 to 3 weeks postexposure, antisera from four E. **canis**-inoculated dogs reacted strongly with the 30-kDa major antigen of E. **canis** but reacted poorly with proteins from E. **chaffeensis**. At 4 weeks postexposure, the sera from three E. ewingii-inoculated dogs showed weak binding to 64- and 47-kDa proteins of both E. **chaffeensis** and E. **canis**. Convalescent-phase sera from human ehrlichiosis patients and sera from dogs chronically infected with E. ewingii strongly reacted with similar sets of proteins of E. **chaffeensis** and E. **canis** with similar intensities. However, sera from dogs chronically infected with E. **canis** reacted more strongly with a greater number of E. **canis** proteins than with E. **chaffeensis** proteins. The protein specificity described in the report suggests that dogs with E. **canis** infections can be distinguished from E. **chaffeensis** -infected animals by **Western immunoblot** analysis with both E. **canis** and E. **chaffeensis** antigens.
AN 1994:407111 BIOSIS
DN PREV199497420111
TI **Western immunoblot** analysis of Ehrlichia **chaffeensis**, E. **canis** or E. ewingii infections in dogs and humans.
AU Rikihisa, Yasuko (1); Ewing, S. A.; Fox, J. C.
CS (1) Dep. Vet. Pathobiol., Coll. Vet. Med., Ohio State Univ., 1925 Coffey Rd., Columbus, OH 43210-1093 USA
SO Journal of Clinical Microbiology, (1994) Vol. 32, No. 9, pp. 2107-2112.
ISSN: 0095-1137.
DT Article
LA English

L8 ANSWER 25 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1994:330641 BIOSIS
DN PREV199497343641
TI Serologic diagnosis of human monocytic ehrlichiosis using **Western immunoblots**: 22-28 kDa immunogenic proteins are species.
AU Brouqui, P. (1); Le Cam, C.; Olson, J.; Raoult, D.
CS (1) Unite Rickettsies, Marseille France
SO Abstracts of the General Meeting of the American Society for Microbiology, (1994) Vol. 94, No. 0, pp. 101.
Meeting Info.: 94th General Meeting of the American Society for

Microbiology Las Vegas, Nevada, USA May 23-27, 1994
ISSN: 1060-2011.
DT Conference
LA English

L8 ANSWER 26 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB Ehrlichia **chaffeensis**, the novel etiologic agent of human ehrlichiosis in the United States, was first isolated in 1990 and reported in 1991. To analyze the antigenic components of E. **chaffeensis**, we cultivated these obligate intracellular bacteria in DH82 cells, purified the ehrlichiae by renografin density gradient centrifugation, and examined the antigens by **Western** immunoblotting. Rabbit and human antisera to E. **chaffeensis** revealed more than 20 bands ranging from 20 to 200 kD. The distinct 22-kD protein was heat labile. The rest of the major immunoreactive components were heat stable. The **immunoblots** of E. **chaffeensis** were highly similar when probed with antisera to E. **chaffeensis**, E. **canis**, and E. ewingii, indicating the close antigenic relationships among the three species. The 22-kD protein cross-reacted only with anti-E. **canis** serum. The antibody against E. sennetsu reacted strongly with the 66-, 64-, 55-, and 44-kD antigens of E. **chaffeensis**. The E. risticii antisera reacted strongly with the 55-and 44-kD bands but only faintly with the 66-kD band. The major immunoreactive antigens of E. **chaffeensis** (66, 55, and 44 kD) showed cross-reactions with all the different antisera tested. The results indicated that E. **chaffeensis** is antigenically most closely related to E. **canis**, is less closely related to E. ewingii, and is only distantly related to E. sennetsu and E. risticii.
AN 1994:161601 BIOSIS
DN PREV199497174601
TI Identification of the antigenic constituents of Ehrlichia **chaffeensis**.
AU Chen, S.-M. (1); Dumler, J. S.; Feng, H.-M. (1); Walker, D. H. (1)
CS (1) Dep. Pathol., Univ. Tex. Med. Branch, 11th St. and Texas Ave., G.129, Galveston, TX 77555-0609 USA
SO American Journal of Tropical Medicine and Hygiene, (1994) Vol. 50, No. 1, pp. 52-58.
ISSN: 0002-9637.
DT Article
LA English

L8 ANSWER 27 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AB An infectious agent was isolated from the enlarged spleen of a wild mouse, Eothenomys kageus, by intraperitoneal inoculation of the spleen homogenate into laboratory mice. The laboratory mice developed splenomegaly, and the agent was maintained by serial passage of spleen homogenates in laboratory mice. The agent in the spleen homogenate was inactivated after incubation at 37 or 50 degree C. Tetracyclines were effective in preventing infection of mice with this agent, but penicillin and sulfonamides were ineffective. Cytoplasmic inclusion bodies were observed in the peritoneal macrophages of infected mice. Electron microscopy revealed numerous small pleomorphic cocci within membrane-lined vacuoles in the cytoplasm of splenic macrophages. Morphologically similar to the ehrlichial organisms, each organism was surrounded by a distinct plasma membrane and rippled outer cell membrane without a distinct peptidoglycan layer. The agent did not grow in chicken embryos, and the Weil-Felix test result was negative. In the indirect fluorescent-antibody test, the agent reciprocally cross-reacted with Ehrlichia **canis** and cross-reacted somewhat with Ehrlichia sennetsu but did not cross-react with Ehrlichia risticii, Neorickettsia helminthoeca, Rickettsia tsutsugamushi, or Chlamydia spp. The mouse antiserum against this agent reacted with 64-, 47-, 46-, 44-, and 40-kDa proteins of E. **canis** by **Western** blotting (immunoblotting). Since E. **canis** and closely related Ehrlichia **chaffeensis** and Ehrlichia ewingii are not known to proliferate or

cause splenomegaly in mice, these results suggest that the agent is a new species within the tribe Ehrlichieae of the family Rickettsiaceae. The finding suggests that wild rodents may serve as reservoirs for pathogenic ehrlichiae.
AN 1993:96148 BIOSIS
DN PREV199395051344
TI Characterization of ehrlichial organisms isolated from a wild mouse.
AU Kawahara, Makoto; Suto, Chiharu; Rikihisa, Yasuko (1); Yamamoto, Seigo; Tsuboi, Yoshimasa
CS (1) Dep. Vet. Pathobiol., Coll. Vet. Med., Ohio State Univ., Columbus, Ohio 43210-1029 USA
SO Journal of Clinical Microbiology, (1993) Vol. 31, No. 1, pp. 89-96. ISSN: 0095-1137.
DT Article
LA English

L8 ANSWER 28 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1992:400852 BIOSIS
DN BR43:56727
TI **WESTERN** BLOT ANALYSIS USING SERA FROM PATIENTS DIAGNOSED WITH HUMAN EHRLICHIOSIS.
AU DAWSON J; GREENE C
CS DIV. VIRAL RICKETTSIAL DISEASES, CENTERS DISEASE CONTROL, ATLANTA, GA.
SO 92ND GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, NEW ORLEANS, LOUISIANA, USA, MAY 26-30, 1992. ABSTR GEN MEET AM SOC MICROBIOL. (1992) 92 (0), 493.
CODEN: AGMME8.
DT Conference
FS BR; OLD
LA English

L8 ANSWER 29 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Canine monocytic ehrlichiosis, caused by Ehrlichia **canis** or Ehrlichia **chaffeensis**, can result in clinical disease in naturally infected animals. Coinfections with these agents may be common in certain areas of endemicity. Currently, a species-specific method for serological diagnosis of monocytic ehrlichiosis is not available. Previously, we developed two indirect enzyme-linked immunosorbent **assays** (**ELISAs**) using the major antigenic protein 2 (MAP2) of E. **chaffeensis** and E. **canis**. In this study, we further characterized the conservation of MAP2 among various geographic isolates of each organism and determined if the recombinant MAP2 (rMAP2) of E. **chaffeensis** would cross-react with E. **canis** -infected dog sera. Genomic Southern blot analysis using digoxigenin-labeled species-specific probes suggested that map2 is a single-copy gene in both Ehrlichia species. Sequences of the single map2 genes of seven geographically different isolates of E. **chaffeensis** and five isolates of E. **canis** are highly conserved among the various isolates of each respective ehrlichial species. **ELISA** and **Western** blot analysis confirmed that the E. **chaffeensis** rMAP2 failed to serologically differentiate between E. **canis** and E. **chaffeensis** infections.
AN 2003:648259 SCISEARCH
GA The Genuine Article (R) Number: 703MU
TI Characterization of the major antigenic protein 2 of Ehrlichia **canis** and Ehrlichia **chaffeensis** and its application for serodiagnosis of ehrlichiosis
AU Knowles T T; Alleman A R (Reprint); Sorenson H L; Marciano D C; Breitschwerdt E B; Harrus S; Barbet A F; Belanger M
CS Univ Florida, Coll Vet Med, Clin Pathol Serv, Dept Physiol Sci, POB 100103, Gainesville, FL 32610 USA (Reprint); Univ Florida, Coll Vet Med, Clin Pathol Serv, Dept Physiol Sci, Gainesville, FL 32610 USA; Univ Florida, Coll Vet Med, Dept Pathobiol, Gainesville, FL 32610 USA; N Carolina State Univ, Coll Vet Med, Dept Compan Anim & Special Species Med,

Raleigh, NC 27606 USA; Hebrew Univ Jerusalem, Sch Vet Med, IL-76100 Rehovot, Israel
CYA USA; Israel
SO CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (JUL 2003) Vol. 10, No. 4, pp. 520-524.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 1071-412X.
DT Article; Journal
LA English
REC Reference Count: 23
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 30 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Objective-To determine historical, physical examination, hematologic, and serologic findings in dogs with Ehrlichia ewingii infection.

Design-Retrospective study.

Animals-15 dogs.

Procedure-In all dogs, infection with E ewingii was confirmed with a polymerase chain reaction (PCR) **assay**. Follow-up information and clarification of information recorded in the medical records was obtained by telephone interviews and facsimile correspondence with referring veterinarians and owners.

Results-Fever and lameness were the most common findings with each occurring in 8 dogs, Five dogs had neurologic abnormalities including ataxia, paresis, proprioceptive deficits, anisocoria, intention tremor, and head tilt. Neutrophilic polyarthritis was identified in 4 dogs. No clinical signs were reported in 3 dogs. The predominant hematologic abnormality was thrombocytopenia, which was identified in all 12 dogs for which a platelet count was available. Reactive lymphocytes were seen in 5 of 13 dogs. Concurrent infection with another rickettsial organism was identified in 4 dogs. Of the 13 dogs tested, 7 were seroreactive to E **canis** antigens. Morulae consistent with E ewingii infection were identified in neutrophils in 8 dogs. Treatment with doxycycline, with or without prednisone, resulted in a rapid, favorable clinical response in the 9 dogs for which follow-up information was available.

Conclusions and Clinical Relevance-Results suggest that PCR testing for E ewingii infection should be considered in dogs with fever, neutrophilic polyarthritis, unexplained ataxia or paresis, thrombocytopenia, or unexplained reactive lymphocytes, and in dogs with clinical signs suggestive of ehrlichiosis that are seronegative for E **canis**. Following treatment with doxycycline, the prognosis for recovery is good.
AN 2003:339084 SCISEARCH
GA The Genuine Article (R) Number: 667VB
TI Molecular identification of Ehrlichia ewingii infection in dogs: 15 cases (1997-2001)
AU Goodman R A; Hawkins E C; Olby N J; Grindem C B; Hegarty B; Breitschwerdt E B (Reprint)
CS N Carolina State Univ, Coll Vet Med, Dept Clin Sci, 4700 Hillsborough St, Raleigh, NC 27606 USA (Reprint); N Carolina State Univ, Coll Vet Med, Dept Clin Sci, Raleigh, NC 27606 USA; N Carolina State Univ, Coll Vet Med, Dept Microbiol, Raleigh, NC 27606 USA; N Carolina State Univ, Coll Vet Med, Dept Parasitol, Raleigh, NC 27606 USA; N Carolina State Univ, Coll Vet Med, Dept Pathol, Raleigh, NC 27606 USA
CYA USA
SO JOURNAL OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION, (15 APR 2003) Vol. 222, No. 8, pp. 1102-1107.
Publisher: AMER VETERINARY MEDICAL ASSOC, 1931 N MEACHAM RD SUITE 100, SCHAUMBURG, IL 60173-4360 USA.
ISSN: 0003-1488.
DT Article; Journal
LA English
REC Reference Count: 28
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 31 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AB A gene encoding a 23.5-kDa ehrlichial morula membrane protein designated MmpA was cloned by screening an Ehrlichia **canis** expression library with convalescent dog sera, which resulted in three positive clones. Sequence analysis of the insert DNAs from all three clones indicated an open reading frame with a size of 666 bp that encodes MmpA. The structural analysis of MmpA indicated that it is a transmembrane protein with extreme hydrophobicity. Southern blot analysis of the HindIII-digested chromosomal DNA demonstrated the presence of a single copy of the mmpA gene in E. **canis** and Ehrlichia **chaffeensis** but not in the human granulocytic ehrlichiosis agent. The mmpA gene was amplified, cloned, and expressed as a fusion protein. Polyclonal antibodies to the recombinant protein (rMmpA) were raised in rabbits. **Western** blot analysis of E. **canis** and E. **chaffeensis** lysates with the anti-rMmpA serum resulted in the presence of an MmpA band only in E. **canis**, not in E. chaffeenesis. Sera from dogs which were either naturally or experimentally infected with E. cams recognized the recombinant protein. Double immunofluorescence confocal microscopy studies demonstrated that MmpA was localized mainly on the morula membrane of E. **canis**. Since the morula membrane is the interface between the ehrlichial growing environment and the host cytoplasm, MmpA may play a role in bacterium-host cell interactions.

AN 2003:311289 SCISEARCH

GA The Genuine Article (R) Number: 662BQ

TI Cloning and characterization of an Ehrlichia **canis** gene encoding a protein localized to the morula membrane

AU Teng C H; Palaniappan R U M; Chang Y F (Reprint)

CS Cornell Univ, Coll Vet Med, Dept Populat Med & Diagnost Sci, Ithaca, NY 14853 USA (Reprint)

CYA USA

SO INFECTION AND IMMUNITY, (APR 2003) Vol. 71, No. 4, pp. 2218-2225. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0019-9567.

DT Article; Journal

LA English

REC Reference Count: 45
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 32 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AB We determined the value of four serological **assays** for the diagnosis of canine monocytic ehrlichiosis by comparing them to the indirect fluorescent-antibody **assay** "gold standard." The specificity of Dip-S-Ticks was significantly lower than that of all of the other tests evaluated. The sensitivity of Dip-S-Ticks was significantly higher than that of Snap3Dx or the Snap Canine Combo. The sensitivity of the rMAP2 enzyme-linked immunosorbent **assay** (**ELISA**) was significantly higher than that of the Snap Canine Combo. The accuracy levels of the rMAP2 **ELISA**, Snap3Dx, Dip-S-Ticks, and Snap Canine Combo were 97.0, 89.8, 85.1, and 82.9%, respectively.

AN 2002:744597 SCISEARCH

GA The Genuine Article (R) Number: 590NX

TI Comparison of serological detection methods for diagnosis of Ehrlichia **canis** infections in dogs

AU Belanger M; Sorenson H L; France M K; Bowie M V; Barbet A F; Breitschwerdt E B; Alleman A R (Reprint)

CS Univ Florida, Coll Vet Med, Dept Physiol Sci, POB 100103, Gainesville, FL 32610 USA (Reprint); Univ Florida, Coll Vet Med, Dept Physiol Sci, Gainesville, FL 32610 USA; Univ Florida, Coll Vet Med, Dept Pathobiol, Gainesville, FL 32610 USA; N Carolina State Univ, Coll Vet Med, Dept Compan Anim & Special Species Med, Raleigh, NC 27606 USA

CYA USA

SO JOURNAL OF CLINICAL MICROBIOLOGY, (SEP 2002) Vol. 40, No. 9, pp. 3506-3508.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0095-1137.
DT Article; Journal
LA English
REC Reference Count: 15
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 33 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB The clinical spectrum of human ehrlichioses ranges from mild febrile illnesses to fatal infections. Laboratory diagnosis of these diseases is based on immunofluorescent **assays** (IFAs) and therefore requires a convalescent serum sample to demonstrate seroconversion or rising antibody titers. More sophisticated techniques, such as **Western** immunoblotting using recombinant proteins, show great promise. Diagnosis during the acute phase, in which IFA is usually nondiagnostic, is based on polymerase chain reaction **assays**. Cultivation of the organisms is difficult and impractical. The treatment of choice is a tetracycline; within this group, doxycycline is the preferred drug because of its better tolerance and lower incidence of side effects.
AN 2002:591186 SCISEARCH
GA The Genuine Article (R) Number: 572YW
TI Human ehrlichioses: Diagnostic challenges and therapeutic recommendations
AU Olano J P (Reprint); Walker D H
CS Univ Texas, Med Branch, WHO Collaborating Ctr Trop Dis, Galveston, TX 77555 USA (Reprint); Univ Texas, Med Branch, Dept Pathol, Galveston, TX 77555 USA
CYA USA
SO INFECTIONS IN MEDICINE, (JUL 2002) Vol. 19, No. 7, pp. 318-325.
Publisher: SCP COMMUNICATIONS INC, 134 W 29TH ST, NEW YORK, NY 10001-5304 USA.
ISSN: 0749-6524.
DT Article; Journal
LA English
REC Reference Count: 49
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 34 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Detection of vector-borne pathogens is necessary for investigation of their association with vertebrate and invertebrate hosts. The ability to detect Ehrlichia spp. within individual experimentally infected ticks would be valuable for studies to evaluate the relative competence of different vector species and transmission scenarios. The purpose of this study was to develop a sensitive PCR **assay** based on oligonucleotide sequences from the unique Ehrlichia **canis** gene, p30, to facilitate studies that require monitoring this pathogen in canine and tick hosts during experimental transmission. Homologous sequences for Ehrlichia **chaffeensis** p28 were compared to sequences of primers derived from a sequence conserved among E. **canis** isolates. Criteria for primer selection included annealing scores, identity of the primers to homologous E. **chaffeensis** sequences, and the availability of similarly optimal primers that were nested within the target template sequence. The p30-based **assay** was at least 100-fold more sensitive than a previously reported nested 16S ribosomal DNA (rDNA)-based **assay** and did not amplify, the 200-bp target amplicon from E. **chaffeensis**, the human granulocytic ehrlichiosis agent, or Ehrlichia muris DNA. The **assay** was used to detect E. **canis** in canine carrier blood and in experimentally infected Rhipicephalus sanguineus ticks. Optimized procedures for preparing tissues from these hosts for PCR **assay** are described. Our results indicated that this p30-based PCR **assay** will be useful for experimental investigations, that it has potential as a routine

test, and that this approach to PCR **assay** design may be applicable to other pathogens that occur at low levels in affected hosts.

AN 2002:128278 SCISEARCH
GA The Genuine Article (R) Number: 519NG
TI Detection of Ehrlichia **canis** in canine carrier blood and in individual experimentally infected ticks with a p30-based PCR **assay**
AU Stich R W (Reprint); Rikihisa Y; Ewing S A; Needham G R; Grover D L; Jittapalapong S
CS Ohio State Univ, Dept Vet Prevent Med, 1900 Coffey Rd, Columbus, OH 43210 USA (Reprint); Ohio State Univ, Dept Vet Prevent Med, Columbus, OH 43210 USA; Ohio State Univ, Dept Vet Biosci, Columbus, OH 43210 USA; Ohio State Univ, Dept Entomol, Columbus, OH 43210 USA; Oklahoma State Univ, Dept Vet Pathobiol, Stillwater, OK 74078 USA
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (FEB 2002) Vol. 40, No. 2, pp. 540-546. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0095-1137.
DT Article; Journal
LA English
REC Reference Count: 37
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 35 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Background: The reported annual incidence of human monocytic ehrlichiosis, which is due to infection with Ehrlichia **chaffeensis**, is as high as 5.5 per million in some states, but serosurveys suggest much higher infection rates in some populations.

Objective: To estimate the prevalence of E **chaffeensis** infection among children aged 1 to 17 years living in the southeast and south-central United States.

Design: Cross-sectional serosurvey.

Setting: Seven academic pediatric medical centers in the southeastern and south-central United States.

Patients: Nineteen hundred ninety-nine children (approximately 300 at each center) having their blood drawn for any reason.

Main Outcome Measure: The presence of antibody at 2 different cutoff titers to E **chaffeensis**, as detected by indirect immunofluorescence **assay**.

Results: Overall, 250 children (13%) had E **chaffeensis** antibody titers of 1:80 or higher and 61 (3%) had titers of 1:160 or higher. Age-adjusted seroprevalence rates varied widely between sites. At 1:80 or higher, the highest rate was in Winston-Salem, NC (22%), and the lowest was in Louisville, Ky (2%). At 1:160 or higher, the highest rate was in Kansas City, Mo (9%), and the lowest was in Oklahoma City, Okla (<1%). In univariate analyses, no associations were found between seroprevalence at either cutoff value and sex, race, source of specimen, or residence demographics. However, age was a significant predictor of seroprevalence at both cutoff values. In multiple logistic regression analysis, study site and age remained strong predictors of seroprevalence, but living in a nonurban ZIP code was not significantly related.

Conclusion: Infection with E **chaffeensis**, or related ehrlichiae, may be more common in children than previously recognized.

AN 2002:120129 SCISEARCH
GA The Genuine Article (R) Number: 518WN
TI Ehrlichia **chaffeensis** seroprevalenee among children in the southeast and south-central regions of the United States
AU Marshall G S (Reprint); Jacobs R F; Schutze G E; Paxton H; Buckingham S C; DeVincenzo J P; Jackson M A; San Joaquin V H; Standaert S M; Woods C R
CS Univ Louisville, Sch Med, Div Pediat Infect Dis, 571 S Floyd St, Suite 321, Louisville, KY 40292 USA (Reprint); Univ Louisville, Sch Med, Div Pediat Infect Dis, Louisville, KY 40292 USA; Univ Arkansas Med Sci, Little Rock, AR 72205 USA; PanBio InDx Inc, Baltimore, MD USA; Univ Tennessee,

Ctr Hlth Sci, Div Pediat Infect Dis, Memphis, TN 38163 USA; Univ Missouri, Div Pediat Infect Dis, Kansas City, MO 64110 USA; Univ Oklahoma, Hlth Sci Ctr, Div Pediat Infect Dis, Oklahoma City, OK USA; Vanderbilt Univ, Sch Med, Dept Prevent Med, Nashville, TN 37212 USA; Wake Forest Univ, Bowman Gray Sch Med, Div Pediat Infect Dis, Winston Salem, NC USA
Corporate Author: Tick-Borne Infections Children Stu
CYA USA
SO ARCHIVES OF PEDIATRICS & ADOLESCENT MEDICINE, (FEB 2002) Vol. 156, No. 2, pp. 166-170.
Publisher: AMER MEDICAL ASSOC, 515 N STATE ST, CHICAGO, IL 60610 USA.
ISSN: 1072-4710.
DT Article; Journal
LA English
REC Reference Count: 32
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 36 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Laboratory diagnosis of human ehrlichioses is routinely made by an indirect immunofluorescence **assay** (IFA) using cultured ehrlichia-infected whole cells as antigen. Concern has been raised that incorrect diagnoses of human monocytic ehrlichiosis (HME) or human granulocytic ehrlichiosis (HGE) may be made on the basis of serologic cross-reactivity between Ehrlichia **chaffeensis** and the agent of HGE. The present study examined whether two recombinant major outer membrane proteins, rP30 and rP44, that were previously shown to be sensitive and specific serodiagnostic antigens for HME and HGE, respectively, could be used to discriminate IFA dually reacting sera. Thirteen dually IFA-reactive sera, three sera that were IFA positive only with E. **chaffeensis**, and three sera that were IFA positive only with the HGE agent were examined by **Western immunoblot** analysis using purified whole organisms and recombinant proteins as antigens. All 16 E. **chaffeensis** IFA-positive sera reacted with rP30. However, none of these sera reacted with rP44, regardless of IFA reactivity with the HGE agent. The three HGE-agent-only IFA-positive sera reacted only with rP44, not with rP30. **Western** immunoblotting using purified E. **chaffeensis** and the HGE agent as antigens suggested that heat shock and other proteins, but not major outer membrane proteins, cross-react between the two organisms. Therefore, **Western immunoblot** analysis using rP44 and rP30 may be useful in discriminating dually HME and HGE IFA-reactive sera.
AN 2001:885150 SCISEARCH
GA The Genuine Article (R) Number: 488KK
TI **Western** blot analysis of sera reactive to human monocytic ehrlichiosis and human granulocytic ehrlichiosis agents
AU Unver A; Felek S; Paddock C D; Zhi N; Horowitz H W; Wormser G P; Cullman L C; Rikihisa Y (Reprint)
CS Ohio State Univ, Coll Vet Med, Dept Vet Biosci, 1925 Coffey Rd, Columbus, OH 43210 USA (Reprint); Ohio State Univ, Coll Vet Med, Dept Vet Biosci, Columbus, OH 43210 USA; Ctr Dis Control & Prevent, Viral & Rickettsial Zoonoses Branch, Atlanta, GA 30333 USA; New York Med Coll, Dept Med, Div Infect Dis, Valhalla, NY 10595 USA; MRL Ref Lab, Cypress, CA 90630 USA
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (NOV 2001) Vol. 39, No. 11, pp. 3982-3986.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0095-1137.
DT Article; Journal
LA English
REC Reference Count: 26
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 37 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB PCR was used to amplify a 537-by region of an Ehrlichia ewingii gene

encoding a homologue of the 28-kDa major antigenic protein (P28) of Ehrlichia **chaffeensis**. The E. ewingii p28 gene homologue was amplified from DNA extracted from whole blood obtained from four humans and one canine with confirmed cases of infection. Sequencing of the PCR products (505 bp) revealed a partial gene with homology to outer membrane protein genes from Ehrlichia and Cowdria spp.: p30 of Ehrlichia cams (less than or equal to 71.3%), p28 of E. **chaffeensis** (less than or equal to 68.3%), and map] of Cowdria ruminantium (67.3%). The peptide sequence of the E. ewingii partial gene product was deduced (168 amino acids) and the antigenicity profile was analyzed, revealing a hydrophilic protein with less than or equal to 69.1% identity to P28 of E. **chaffeensis**, less than or equal to 67.3% identity to P30 of E. **canis**, and less than or equal to 63.1% identity to MAPI of C. ruminantium. Primers were selected from the E. ewingii p28 sequence and used to develop a species-specific PCR diagnostic **assay**. The p28 PCR **assay** amplified the expected 215-bp product from DNA that was extracted from EDTA-treated blood from each of the confirmed E. ewingii infections that were available. The **assay** did not produce PCR products with DNA extracted from E. **chaffeensis**-, E. **canis**-, or E. phagocytophila-infected samples, confirming the specificity of the p28 **assay** for E. ewingii. The sensitivity of the E. ewingii-specific PCR **assay** was evaluated and determined to detect as few as 38 copies of the p28 gene.

AN 2001:885132 SCISEARCH
GA The Genuine Article (R) Number: 488KK
TI Identification of a p28 gene in Ehrlichia ewingii: Evaluation of gene for use as a target for a species-specific PCR diagnostic **assay**
AU Gusa A A; Buller R S; Storch G A; Huycke M M; Machado L J; Slater L N; Stockham S L; Massung R F (Reprint)
CS CDCP, Div Viral & Rickettsial Dis, Natl Ctr Infect Dis, 1600 Clifton Rd, MS G-13, Atlanta, GA 30333 USA (Reprint); CDCP, Div Viral & Rickettsial Dis, Natl Ctr Infect Dis, Atlanta, GA 30333 USA; Washington Univ, Sch Med, Edward Mallinckrodt Dept Pediat, St Louis, MO 63110 USA; St Louis Childrens Hosp, St Louis, MO 63178 USA; Univ Missouri, Coll Vet Med, Dept Vet Pathobiol, Columbia, MO USA; Univ Oklahoma, Hlth Sci Ctr, Dept Med, Div Infect Dis, Oklahoma City, OK USA; Dept Vet Affairs Med Ctr, Oklahoma City, OK USA
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (NOV 2001) Vol. 39, No. 11, pp. 3871-3876.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0095-1137.
DT Article; Journal
LA English
REC Reference Count: 28
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 38 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Forty-nine dogs from Thailand were evaluated for serologic evidence of exposure or polymerase chain reaction (PCR) evidence of infection with vectorborne pathogens, including Ehrlichia sp. (Ehrlichia **canis**, Ehrlichia **chaffeensis**, Ehrlichia equi, and Ehrlichia risticii). Bartonella vinsonii subsp. berkhoffi (Bvb), spotted fever group (SFG) rickettsiae (Rickettsia rickettsii), Typhus group (TG) rickettsiae (Rickettsia canada, Rickettsia prowazekii. and Rickettsia typhi). and Babesia sp. (Bahesia **canis** and Babesia gibsonii). All study dogs had at least I of 3 entry criteria: fever, anemia, or thrombocytopenia, By immunofluorescence antibody (IFA) testing, seroreactivity was most prevalent to E **chaffeensis** (74%) and E cams (71%) antigens, followed by E equi (.58%), Bvb (38%), E risticii (38%). R prowazekii (24%), B **canis** (20%). R rickettsii (12%), R canada (4%), and B gibsonii (4%) antigens. There was 100% concordance between E **canis** IFA and **western** blot **immunoassay** (WI) for 35 of 35

samples 2 samples were IFA and WI reactive only to E equi antigens. By PCR amplification, 10 dogs were found to be infected with E **canis**, 5 with Ehrlichia platys, and 3 with B **canis**. Sequencing of PCR products was undertaken to compare Ehrlichia strains from Thailand to strains originating from the United States. Partial DNA sequence analysis confirmed infection with E **canis** and E platys, with identical 16S rRNA sequence alignment to E **canis** (U26740) and to E platys (M83801), as reported in GenBank. Partial E **canis** P28.1 and P28.2 amino acid sequences from Thai dogs were divergent from analogous sequences derived from North American E **canis** (AF082744) strains, suggesting that the Thai dogs were infected with a geographically distinct strain of E **canis** compared to North American strains. The results of this study indicate that dogs in Thailand have substantial exposure to vectorborne diseases and that coinfection with these pathogens may be common.

AN 2001:771051 SCISEARCH

GA The Genuine Article (R) Number: 474FT

TI Serologic and molecular evidence of coinfection with multiple vector-borne pathogens in dogs from Thailand

AU Suksawat J; Yu X J; Hancock S I; Hegarty B C; Nilkumhang P; Breitschwerdt E B (Reprint)

CS N Carolina State Univ, Coll Vet Med, Dept Clin Sci, 4700 Hillsborough St, Raleigh, NC 27606 USA (Reprint); N Carolina State Univ, Coll Vet Med, Dept Clin Sci, Raleigh, NC 27606 USA; Khon Kaen Univ, Fac Vet Med, Dept Vet Med, Khon Kaen, Thailand; Univ Texas, Med Branch, Sch Med, Dept Pathol, Galveston, TX 77550 USA; Kasetsart Univ, Fac Vet Med, Dept Small Anim Med, Bangkok, Thailand

CYA USA; Thailand

SO JOURNAL OF VETERINARY INTERNAL MEDICINE, (SEP-OCT 2001) Vol. 15, No. 5, pp. 453-462.
Publisher: AMER COLL VETERINARY INTERNAL MEDICINE, 7175 W JEFFERSON AVE, STE 2125, LAKEWOOD, CO 80235 USA.
ISSN: 0891-6640.

DT Article; Journal

LA English

REC Reference Count: 68
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 39 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AB Ehrlichia **canis**, an obligatory intracellular bacterium of monocytes and macrophages, causes canine monocytic ehrlichiosis. E. **canis** immunodominant 30-kDa major outer membrane proteins are encoded by a polymorphic multigene family consisting of more than 20 paralogs. In the present study, we analyzed the mRNA expression of 14 paralogs in experimentally infected dogs and Rhipicephalus sanguineus ticks by reverse transcription-PCR using gene-specific primers followed by Southern blotting. Eleven out of 14 paralogs in E. **canis** were transcribed in increasing numbers and transcription levels, while the mRNA expression of the 3 remaining paralogs was not detected in blood monocytes of infected dogs during the 56-day postinoculation period. Three different groups of R. sanguineus ticks (adult males and females and nymphs) were separately infected with E. **canis** by feeding on the infected dogs. In these pools of acquisition-fed ticks as well as in the transmission-fed adult ticks, the transcript from only one paralog was detected, suggesting the predominant transcription of that paralog or the suppression of the remaining paralogs in ticks. Expression of the same paralog was higher whereas expression of the remaining paralogs was lower in E. **canis** cultivated in dog monocyte cell line DH82 at 25 degreesC than in E. **canis** cultivated at 37 degreesC. Analysis of differential expression of p30 multigenes in dogs, ticks, or monocyte cell cultures would help in understanding the role of these gene products in pathogenesis and E. **canis** transmission as well as in designing a rational vaccine candidate immunogenic against canine ehrlichiosis.

AN 2001:764483 SCISEARCH

GA The Genuine Article (R) Number: 474RR
TI Transcriptional analysis of p30 major outer membrane multigene family of Ehrlichia **canis** in dogs, ticks, and cell culture at different temperatures
AU Unver A; Ohashi N; Tajima T; Stich R W; Grover D; Rikihisa Y (Reprint)
CS Ohio State Univ, Coll Vet Med, Dept Vet Biosci, 1925 Coffey Rd, Columbus, OH 43210 USA (Reprint); Ohio State Univ, Coll Vet Med, Dept Vet Biosci, Columbus, OH 43210 USA; Ohio State Univ, Coll Vet Med, Dept Vet Prevent Med, Columbus, OH 43210 USA
CYA USA
SO INFECTION AND IMMUNITY, (OCT 2001) Vol. 69, No. 10, pp. 6172-6178.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0019-9567.
DT Article; Journal
LA English
REC Reference Count: 32
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 40 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB We previously culture isolated a strain of Ehrlichia **canis**, the causative agent of canine ehrlichiosis, from a human in Venezuela. In the present study, we examined whether dogs and ticks are infected with E. **canis** in Venezuela and, if so, whether this is the same strain as the human isolate. PCR analysis using E. **canis**-specific primers revealed that 17 of the 55 dog blood samples (31%) and all three pools of four Rhipicephalus sanguineus ticks each were positive. An ehrlichial agent (Venezuelan dog Ehrlichia [VDE]) was isolated and propagated in cell culture from one dog sample and was further analyzed to determine its molecular and antigenic characteristics. The 16S rRNA 1,408-bp sequence of the new VDE isolate was identical to that of the previously reported Venezuelan human Ehrlichia isolate (VHE) and was closely related (99.9%) to that of E. **canis** Oklahoma. The 5' (333-bp) and 3' (653-bp) sequences of the variable regions of the 16S rRNA genes from six additional E. **canis**-positive dog blood specimens and from three pooled-tick specimens were also identical to those of VHE. **Western** blot analysis of serum samples from three dogs infected with VDE by using several ehrlichial antigens revealed that the antigenic profile of the VDE was similar to the profiles of VHE and E. **canis** Oklahoma. Identical 16S rRNA gene sequences among ehrlichial organisms from dogs, ticks, and a human in the same geographic region in Venezuela and similar antigenic profiles between the dog and human isolates suggest that dogs serve as a reservoir of human E. **canis** infection and that R. sanguineus, which occasionally bites humans residing or traveling in this region, serves as a vector. This is the first report of culture isolation and antigenic characterization of an ehrlichial agent from a dog in South America, as well as the first molecular characterization of E. **canis** directly from naturally infected ticks.
AN 2001:624825 SCISEARCH
GA The Genuine Article (R) Number: 459HT
TI Molecular and antigenic comparison of Ehrlichia **canis** isolates from dogs, ticks, and a human in Venezuela
AU Unver A; Perez M; Orellana N; Huang H B; Rikihisa Y (Reprint)
CS Ohio State Univ, Coll Vet Med, Dept Vet Biosci, 1925 Coffey Rd, Columbus, OH 43210 USA (Reprint); Ohio State Univ, Coll Vet Med, Dept Vet Biosci, Columbus, OH 43210 USA; Univ Centroccidental Lisandro Alvarado, Dept Med Cirugia, Tarabana, Venezuela
CYA USA; Venezuela
SO JOURNAL OF CLINICAL MICROBIOLOGY, (AUG 2001) Vol. 39, No. 8, pp. 2788-2793.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0095-1137.
DT Article; Journal

LA English
REC Reference Count: 32
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 41 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB The major antigenic protein 2 (MAP2) of Ehrlichia **canis** was cloned and expressed. The recombinant protein was characterized and tested in an enzyme-linked immunosorbent **assay** (**ELISA**) format for potential application in the serodiagnosis of canine monocytic ehrlichiosis. The recombinant protein, which contained a C-terminal polyhistidine tag, had a molecular mass of approximately 26 kDa, The antigen was clearly identified by **Western** immunoblotting using antihistidine antibody and immune serum from an experimentally infected dog. The recombinant MAP2 (rMAP2) was tested in an **ELISA** format using 141 serum samples from E. **canis** immunofluorescent antibody (IFA)-positive and IFA-negative dogs. Fifty-five of the serum samples were from dogs experimentally or naturally infected with E. **canis** and were previously demonstrated to contain antibodies reactive with E. **canis** by indirect immunofluorescence **assays**. The remaining 86 samples, 33 of which were from dogs infected with microorganisms other than E. **canis**, were seronegative. All of the samples from experimentally infected animals and 36 of the 37 samples from naturally infected animals were found to contain antibodies against rMAP2 of E. **canis** in the **ELISA**. Only 3 of 53 IFA-negative samples tested positive on the rMAP2 **ELISA**. There was 100% agreement among IFA-positive samples from experimentally infected animals, 97.3% agreement among IFA-positive samples from naturally infected animals, and 94.3% agreement among IFA-negative samples, resulting in a 97.2% overall agreement between the two **assays**. These data suggest that rMAP2 of e. **canis** could be used as a recombinant test antigen for the serodiagnosis of canine monocgtic ehrlichiosis.
AN 2001:546318 SCISEARCH
GA The Genuine Article (R) Number: 447RN
TI Recombinant major antigenic protein 2 of Ehrlichia **canis**: A potential diagnostic tool
AU Alleman A R (Reprint); McSherry L J; Barbet A F; Breitschwerdt E B; Sorenson H L; Bowie M V; Belanger M
CS Univ Florida, Coll Vet Med, Dept Physiol Sci, Box 100103C, Gainesville, FL 32610 USA (Reprint); Univ Florida, Coll Vet Med, Dept Physiol Sci, Gainesville, FL 32610 USA; Univ Florida, Coll Vet Med, Dept Pathobiol, Gainesville, FL 32610 USA; N Carolina State Univ, Coll Vet Med, Dept Compan Anim & Special Species Med, Raleigh, NC 27606 USA
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (JUL 2001) Vol. 39, No. 7, pp. 2494-2499.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0095-1137.
DT Article; Journal
LA English
REC Reference Count: 38
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 42 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Ehrlichia **chaffeensis** is an obligatory intracellular bacterium of monocytes and macrophages and the etiologic agent of human monocytic ehrlichiosis, an emerging zoonosis. The Lone Star tick (Amblyomma americanum) has been implicated as the primary vector of E. **chaffeensis**. The present study examined the sensitivity of the nested reverse transcription (RT)-PCR based on the 16S rRNA gene relative to that of the nested PCR for detection of E. **chaffeensis** in infected DH82 cells, experimentally infected dog peripheral blood mononuclear cells, or experimentally infected A. americanum tick samples.

The RT-PCR was found to be approximately 100 times more sensitive than the PCR for detection of E. **chaffeensis** regardless of the nature of the specimens. Thus, this RT-PCR is useful for detection off. **chaffeensis** when a high sensitivity is required. Positive results by RT-PCR also imply the presence of viable pathogens. This is the first demonstration of RNA of E. **chaffeensis** in infected blood and acquisition-fed male, nymphal, and larval A. americanum ticks.

AN 2001:145035 SCISEARCH
GA The Genuine Article (R) Number: 398VA
TI Sensitive detection of Ehrlichia **chaffeensis** in cell culture, blood, and tick specimens by reverse transcription-PCR
AU Felek S; Unver A; Stich R W; Rikihisa Y (Reprint)
CS Ohio State Univ, Coll Vet Med, Dept Vet Biosci, 1925 Coffey Rd, Columbus, OH 43210 USA (Reprint); Ohio State Univ, Coll Vet Med, Dept Vet Biosci, Columbus, OH 43210 USA; Ohio State Univ, Coll Vet Med, Dept Vet Prevent Med, Columbus, OH 43210 USA
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (FEB 2001) Vol. 39, No. 2, pp. 460-463. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0095-1137.
DT Article; Journal
LA English
REC Reference Count: 27
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 43 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Ehrlichia **canis** causes a potentially fatal rickettsial disease of dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. We recently reported the cloning of two immunoreactive E. **canis** proteins, P28 and P140, that were applicable for serodiagnosis of the disease. In the present study we cloned a new immunoreactive E. **canis** surface protein gene of 1,170 bp, which encodes a protein with a predicted molecular mass of 42.6 kDa (P43). The P43 gene was not detected in E. **chaffeensis** DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. **chaffeensis** as detected by indirect fluorescent antibody (IFA) **assay**. Forty-two dogs exhibiting signs and/or hematologic abnormalities associated with canine ehrlichiosis were tested by IFA **assay** and by recombinant **Western immunoblot**. hmong the 22 samples that were IFA positive for E. **canis**, 100% reacted with rP43, 96% reacted with rP28, and 96% reacted with rP140. The specificity of the recombinant proteins compared to the IFAs was 96% for rP28, 88% for P43 and 63% for P140. The results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for E. **canis** infections.

AN 2001:84115 SCISEARCH
GA The Genuine Article (R) Number: 393KZ
TI Immunodiagnosis of Ehrlichia **canis** infection with recombinant proteins
AU McBride J W; Corstvet R E; Breitschwerdt E B; Walker D H (Reprint)
CS Univ Texas, Med Branch, Dept Pathol, 301 Univ Blvd, Galveston, TX 77555 USA (Reprint); Univ Texas, Med Branch, Dept Pathol, Galveston, TX 77555 USA; Univ Texas, Med Branch, WHO, Collaborating Ctr Trop Dis, Galveston, TX 77555 USA; Louisiana State Univ, Sch Vet Med, Dept Vet Microbiol & Parasitol, Baton Rouge, LA 70803 USA; N Carolina State Univ, Coll Vet Med, Dept Compan Anim & Special Species Med, Raleigh, NC 27606 USA
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (JAN 2001) Vol. 39, No. 1, pp. 315-322. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0095-1137.
DT Article; Journal

LA English
REC Reference Count: 29
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 44 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB A nested polymerase chain reaction **assay** was used to determine the presence of Ehrlichia **chaffeensis**, E. **canis**, and E. ewingii DNA in blood samples of free-ranging coyotes from central and northcentral Oklahoma. Of the 21 coyotes examined, 15 (71%) were positive for E. **chaffeensis** DNA; none was positive for E. **canis** or E. ewingii. Results suggest that E. **chaffeensis** infections are common in free-ranging coyotes in Oklahoma and that these wild canids could play a role in the epidemiology of human monocytotropic ehrlichiosis.
AN 2000:777204 SCISEARCH
GA The Genuine Article (R) Number: 362PA
TI Naturally occurring Ehrlichia **chaffeensis** infection in coyotes from Oklahoma
AU Kocan A (Reprint); Levesque G C; Whitworth L C; Murphy G L; Ewing S A; Barker R W
CS OKLAHOMA STATE UNIV, COLL VET MED, DEPT VET PATHOBIOL, STILLWATER, OK 74078 (Reprint)
CYA USA
SO EMERGING INFECTIOUS DISEASES, (SEP-OCT 2000) Vol. 6, No. 5, pp. 477-480. Publisher: CENTER DISEASE CONTROL, ATLANTA, GA 30333. ISSN: 1080-6040.
DT Article; Journal
FS CLIN
LA English
REC Reference Count: 28
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 45 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB The major antigenic protein 2 (MAP2) homolog of Ehrlichia **chaffeensis** was cloned and expressed. The recombinant protein was characterized and tested in an enzyme-linked immunosorbent **assay** (**ELISA**) format for potential application in the serodiagnosis of human monocytic ehrlichiosis. The recombinant protein, which contained a C-terminal polyhistidine tag, had a molecular mass of approximately 26 kDa, The antigen was clearly identified by **Western** immunoblotting using antihistidine antibody. However, immune sera failed to react with the recombinant on **immunoblots** when the antigen was denatured by heat or reduced using beta-mercaptoethanol. The recombinant MAP2, (rMAP2) was used in an **ELISA** format with 60 blinded serum samples. Twenty of the serum samples were previously demonstrated to contain antibodies reactive with E. **chaffeensis** by indirect immunofluorescence **assays** (IFAs), The remaining 40 samples were seronegative. All samples negative by IFA were also found to be negative for antibodies against the rMAP2 of E. **chaffeensis** by using the **ELISA**, Only 1 of 20 IFA-positive samples tested negative in the rMAP2 **ELISA**. There was 100% agreement using HA-negative samples and 95% agreement using IFA-positive samples, resulting in a 97.5% overall agreement between the two **assays**. These data suggest that the rMAP2 homolog of E. **chaffeensis** may have potential as a test antigen for the serodiagnosis of human monocytic ehrlichiosis. To our knowledge, this recombinant is unique because it is thus far the only E. **chaffeensis** recombinant antigen that has been shown to work in an **ELISA** format.
AN 2000:766684 SCISEARCH
GA The Genuine Article (R) Number: 361DP
TI Expression of a gene encoding the major antigenic protein 2 homolog of Ehrlichia **chaffeensis** and potential application for serodiagnosis
AU Alleman A R (Reprint); Barbet A F; Bowie M V; Sorenson H L; Wong S J;

Belanger M
CS UNIV FLORIDA, COLL VET MED, DEPT PHYSIOL SCI, BOX 100103C, GAINESVILLE, FL 32610 (Reprint); UNIV FLORIDA, COLL VET MED, DEPT PATHOBIOL, GAINESVILLE, FL 32610; NEW YORK STATE DEPT HLTH, WADSWORTH CTR, ALBANY, NY 12201
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (OCT 2000) Vol. 38, No. 10, pp. 3705-3709.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904.
ISSN: 0095-1137.
DT Article; Journal
FS LIFE; CLIN
LA English
REC Reference Count: 22
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 46 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Detection of Ehrlichia **canis** in acutely infected and convalescent dogs is important for effective treatment and control. However, accurate detection has been difficult to achieve, in part because dogs that have been treated therapeutically often remain seropositive for extended periods. A new method, polymerase chain reaction (PCR) **assay** using biotinylated E. **canis**-specific primers (PCR-BP), was developed for detection of E. **canis**. Four dogs experimentally infected with E. **canis** by intravenous inoculation of whole blood from carrier dogs and 2 naturally infected convalescent carriers were used to compare the specificity and sensitivity of the new method with that of microscopy/blood smear evaluation, serologic test, and conventional PCR **assay** using E. **canis**-specific primers. In experimentally infected animals, infection was detected as early as 7 days postexposure using PCR-BP. Although the 2 naturally infected dogs were positive by serologic test and PCR-BP, both were negative by conventional PCR. Results suggest that the new method is a sensitive **assay** for detection of E. **canis** infection. In addition, results were obtained more rapidly than with other PCR-based **assays**.
AN 2000:724719 SCISEARCH
GA The Genuine Article (R) Number: 355VE
TI Efficacy of a modified polymerase chain reaction **assay** for detection of Ehrlichia **canis** infection
AU Mathew J S (Reprint); Ewing S A; Malayer J R; Fox J C; Kocan K M
CS HARVARD UNIV, SCH MED, NERPRC, POB 9102, SOUTHBOROUGH, MA 01772 (Reprint); OKLAHOMA STATE UNIV, COLL VET MED, STILLWATER, OK 74078
CYA USA
SO JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (SEP 2000) Vol. 12, No. 5, pp. 456-459.
Publisher: AMER ASSOC VETERINARY LABORATORY DIAGNOSTICIANS INC, PO BOX 1522, TURLOCK, CA 95381.
ISSN: 1040-6387.
DT Article; Journal
FS AGRI
LA English
REC Reference Count: 14
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 47 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Red foxes (Vulpes vulpes) and gray foxes (Urocyon cinereoargenteus) were evaluated for their susceptibility to experimental infection with Ehrlichia **chaffeensis**, the causative agent of human monocytotropic ehrlichiosis. Two red foxes and three gray foxes were inoculated intravenously with E. **chaffeensis** (15B-WTD-GA strain) and were monitored at. 7, 14, 21, and 28 days post inoculation (DPI) for evidence of infection using an indirect fluorescent antibody (IFA) **assay**, light microscopy, polymerase chain reaction (PCR), and cell culture methods. One red fox and one gray fox served as negative controls.

Red foxes were susceptible to infection based on reisolation of E. **chaffeensis** from blood at 7 and 14 DPI, seroconversion by 7 DPI, and positive PCR **assays** on spleen and lymph nodes at 28 DPI. Morulae were not found in circulating leukocytes and clinical signs or lesions of ehrlichiosis were not observed. In contrast, gray foxes were refractory to infection based on negative results on all culture, PCR, serologic, and microscopic examinations. These findings imply that red foxes, but not gray foxes, are potential vertebrate reservoirs for E. **chaffeensis**. These findings also illustrate the need to verify serologic evidence of E. **chaffeensis** infection among wild animals.

AN 2000:439763 SCISEARCH
GA The Genuine Article (R) Number: 321VL
TI Susceptibility of red and gray foxes to infection by Ehrlichia **chaffeensis**
AU Davidson W R (Reprint); Lockhart J M; Stallknecht D E; Howerth E W
CS UNIV GEORGIA, COLL VET MED, SE COOPERAT WILDLIFE DIS STUDY, ATHENS, GA 30602 (Reprint); UNIV GEORGIA, WARNELL SCH FOREST RESOURCES, ATHENS, GA 30602; UNIV GEORGIA, COLL VET MED, DEPT PATHOL, ATHENS, GA 30602
CYA USA
SO JOURNAL OF WILDLIFE DISEASES, (OCT 1999) Vol. 35, No. 4, pp. 696-702. Publisher: WILDLIFE DISEASE ASSN, INC, 810 EAST 10TH ST, LAWRENCE, KS 66044-8897.
ISSN: 0090-3558.
DT Article; Journal
FS AGRI
LA English
REC Reference Count: 35
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 48 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Ehrlichia **canis**, E equi, and E risticii seroprevalence was determined by microimmunofluorescent antibody testing (IFA) in a sequential population of 1,845 sick dogs admitted during a 1-year period to the North Carolina Stars University Veterinary Teaching Hospital. A seroreactor was defined by a reciprocal IFA titer of greater than or equal to 80 to E **canis**, E equi, or E risticii antigens. Of the 48 IFA seroreactors. 44 dogs were seroreactive to E **canis**, 21 to E equi, and 0 to E risticii. Seventeen dogs reacted to both E **canis** and E equi antigens. There was concordance of E **canis** IFA and **western immunoblot** (WI) test results fur 36/44 dogs. Because of cross-reactivity of E **canis** sera with E equi antigens, WI was of less utility to confirm E equi exposure. After elimination of E **canis** seroreactors, there was concordance of 2/4 E equi IFA and WI test results. Based upon a retrospective review of medical records, ehrlichiosis was diagnosed in 10/48 (21%) IFA seroreactive dogs, 9 of which were confirmed positive by WI. Of the remaining 38 IFA seroreactors, 29 also were confirmed by E **canis** or E equi WI. These results indicate that (1) ehrlichiosis nor diagnosed in the majority of serologically confirmed cases, (2) based upon E **canis** and E equi WI analysis, IFA testing was not specific (21% false positive). (3) E **canis** sera cross-react with E equi antigens, and (4) serologic evidence of E risticii infection was lacking in the dog population studied.

AN 2000:102749 SCISEARCH
GA The Genuine Article (R) Number: 279UM
TI Seroprevalence of Ehrlichia **canis**, Ehrlichia equi, and Ehrlichia risticii in sick dogs from North Carolina and Virginia
AU Suksawat J; Hegarty B C; Breitschwerdt E B (Reprint)
CS N CAROLINA STATE UNIV, COLL VET MED, DEPT CLIN SCI, 4700 HILLSBOROUGH ST, RALEIGH, NC 27606 (Reprint); N CAROLINA STATE UNIV, COLL VET MED, DEPT CLIN SCI, RALEIGH, NC 27606
CYA USA
SO JOURNAL OF VETERINARY INTERNAL MEDICINE, (JAN-FEB 2000) Vol. 14, No. 1,

pp. 50-55.
Publisher: AMER COLL VETERINARY INTERNAL MEDICINE, 7175 W JEFFERSON AVE, STE 2125, LAKEWOOD, CO 80235.
ISSN: 0891-6640.
DT Article; Journal
FS AGRI
LA English
REC Reference Count: 36
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 49 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB We conducted a retrospective serosurvey of 1,000 persons in Israel who had fever of undetermined cause to look for Ehrlichia **chaffeensis** antibodies. Four of five cases with antibodies reactive to E. **chaffeensis** were diagnosed in the summer, when ticks are more active. All patients had influenzalike symptoms with high fever. None of the cases was fatal. Three serum samples were also seroreactive for antibodies to E. **canis**, and one was also reactive to the human granulocytic ehrlichiosis (HGE) agent. The titer to the HGE agent in this patient was higher than the serum titer to E. **chaffeensis**, and the **Western** blot analysis also indicated that the HGE agent was the primary cause of infection. We present the first serologic evidence that the agents of human monocytic ehrlichiosis (HME) and HGE are present in Israel. Therefore, human ehrlichiosis should be included in the differential diagnoses for persons in Israel who have been exposed to ticks and have influenzalike symptoms.
AN 2000:16939 SCISEARCH
GA The Genuine Article (R) Number: 268HK
TI Serologic evidence of human monocytic and granulocytic ehrlichiosis in Israel
AU Keysary A; Amram L; Keren G; Sthoeger Z; Potasman I; Jacob A; Strenger C; Dawson J E; Waner T (Reprint)
CS ISRAEL INST BIOL RES, POB 19, IL-70400 NESS ZIONA, ISRAEL (Reprint); ISRAEL INST BIOL RES, IL-70400 NESS ZIONA, ISRAEL; ASAF HAROFE MED CTR, ZERIFIN, ISRAEL; CHAIM SHEBA MED CTR, IL-52621 TEL HASHOMER, ISRAEL; KAPLAN HOSP, IL-76100 REHOVOT, ISRAEL; BNEI ZION MED CTR, HAIFA, ISRAEL; SCHNEIDER CHILDRENS MED CTR ISRAEL, PETAH TIQWA, ISRAEL; CTR DIS CONTROL & PREVENT, ATLANTA, GA
CYA ISRAEL; USA
SO EMERGING INFECTIOUS DISEASES, (NOV-DEC 1999) Vol. 5, No. 6, pp. 775-778.
Publisher: CENTER DISEASE CONTROL, ATLANTA, GA 30333.
ISSN: 1080-6040.
DT Article; Journal
FS CLIN
LA English
REC Reference Count: 13
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 50 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Human monocytic ehrlichiosis is an emerging infectious disease caused by Ehrlichia **chaffeensis**, a gramnegative obligatory intracellular bacterium closely related to E, **canis**, The immunoreactive recombinant fusion proteins rP28 and rP30 have become available after cloning and expressing of the 28- and 30-kDa major outer membrane protein genes of E. **chaffeensis** and E, **canis**, respectively. **Western** immunoblotting was performed to analyze the antibody responses of the 37 E. **chaffeensis** indirect fluorescent-antibody **assay** (IFA)-positive and 20 IFA-negative serum specimens with purified whole organisms, rP28, and rP30, All IFA-negative sera were negative with purified whole organisms, rP28, or rP30 by **Western immunoblot** analysis (100% relative diagnostic specificity). Of 37 IFA-positive sera, 34 sera reacted with any native proteins of E. **chaffeensis** ranging from 44 to 110 kDa, and 30 sera reacted with 44- to 110-kDa native E, **canis**

antigens. The 28-kDa E. **chaffeensis** and 30-kDa E. **canis** native proteins were recognized by 25 IFA-positive sera, Fifteen IFA-positive sera reacted with rP28 by **Western** blot analysis, whereas 34 IFA-positive sera reacted with rP30 (92% relative diagnostic specificity), indicating that rP30 is more sensitive than rP28 for detecting the antibodies in IFA-positive sera. These 34IFA-positive sera were positive by the dot blot **assay** with rP30, distinguishing them from IFA-negative sera. Except for three rP30-negative but IFA-positive specimens that instead showed an E. ewingii infection-like profile by **Western** immunoblotting, the results of **Western** and dot blot **assays** with rP30 matched 100% with the LFA test results. Densitometric analysis of dot blot reactions showed a positive correlation between the dot density and the IFA titer, These results suggest that rP30 antigen would provide a simple, consistent, and rapid serodiagnosis for human monocytic ehrlichiosis.

AN 1999:926161 SCISEARCH
GA The Genuine Article (R) Number: 259CY
TI **Western** and dot blotting analyses of Ehrlichia **chaffeensis** indirect fluorescent-antibody **assay**-positive and -negative human sera by using native and recombinant E. **chaffeensis** and E. **canis** antigens
AU Unver A; Rikihisa Y (Reprint); Ohashi N; Cullman L C; Buller R; Storch G A
CS OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, 1925 COFFEY RD, COLUMBUS, OH 43210 (Reprint); OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, COLUMBUS, OH 43210; WASHINGTON UNIV, SCH MED, ST LOUIS, MO 63110; MRL REFERENCE LAB, CYPRESS, CA 90630
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (DEC 1999) Vol. 37, No. 12, pp. 3888-3895.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0095-1137.
DT Article; Journal
FS LIFE; CLIN
LA English
REC Reference Count: 35
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 51 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Background Human ehrlichiosis is a recently recognized tick-borne infection. Four species infect humans: Ehrlichia **chaffeensis**, E. sennetsu, E. **canis**, and the agent of human granulocytic ehrlichiosis.

Methods We tested peripheral-blood leukocytes from 413 patients with possible ehrlichiosis by broad-range and species-specific polymerase-chain-reaction (PCR) **assays** for ehrlichia. The species present were identified by species-specific PCR **assays** and nucleotide sequencing of the gene encoding ehrlichia 16S ribosomal RNA. **Western** blot analysis was used to study serologic responses.

Results In four patients, ehrlichia DNA was detected in leukocytes by a broad-range PCR **assay**, but not by **assays** specific for E. **chaffeensis** or the agent of human granulocytic ehrlichiosis. The nucleotide sequences of these PCR products matched that of E. ewingii, an agent previously reported as a cause of granulocytic ehrlichiosis in dogs. These four patients, all from Missouri, presented between May and August 1996, 1997, or 1998 with fever, headache, and thrombocytopenia, with or without leukopenia. All had been exposed to ticks, and three were receiving immunosuppressive therapy. Serum samples obtained from three of these patients during convalescence contained antibodies that reacted with E, **chaffeensis** and E. **canis** antigens in a pattern different from that of humans with E. **chaffeensis** infection but similar to that of a dog experimentally infected with E. ewingii. Morulae were identified in neutrophils from two patients. All four patients were

successfully treated with doxycycline.
Conclusions These findings provide evidence of E. ewingii infection in humans. The associated disease may be clinically indistinguishable from infection caused by E. **chaffeensis** or the agent of human granulocytic ehrlichiosis. (N Engl J Med 1999;341: 148-55.) (C) 1999, Massachusetts Medical Society.

AN 1999:549705 SCISEARCH
GA The Genuine Article (R) Number: 215YV
TI Ehrlichia ewingii, A newly recognized agent of human ehrlichiosis
AU Buller R S; Arens M; Hmiel S P; Paddock C D; Sumner J W; Rikihisa Y; Unver A; GaudreaultKeener R; Manian F A; Liddell A M; Schmulewitz N; Storch G A (Reprint)
CS ST LOUIS CHILDRENS HOSP, DEPT PEDIAT, DIV INFECT DIS, 1 CHILDRENS PL, ST LOUIS, MO 63110 (Reprint); ST LOUIS CHILDRENS HOSP, DEPT PEDIAT, DIV INFECT DIS, ST LOUIS, MO 63110; WASHINGTON UNIV, SCH MED, EDWARD MALLINCKRODT DEPT PEDIAT, ST LOUIS, MO 63110; WASHINGTON UNIV, SCH MED, DEPT MED, ST LOUIS, MO 63110; CTR DIS CONTROL & PREVENT, ATLANTA, GA; OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, COLUMBUS, OH 43210; ST JOHNS MERCY MED CTR, ST LOUIS, MO 63141
CYA USA
SO NEW ENGLAND JOURNAL OF MEDICINE, (15 JUL 1999) Vol. 341, No. 3, pp. 148-155.
Publisher: MASSACHUSETTS MEDICAL SOC, WALTHAM WOODS, 860 WINTER ST, WALTHAM, MA 02451-1413.
ISSN: 0028-4793.
DT Article; Journal
FS LIFE; CLIN
LA English
REC Reference Count: 31
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 52 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Ehrlichia **chaffeensis** is an obligatory intracellular bacterium that infects the monocyte-macrophage. E. **chaffeensis**, which is transmitted to humans by ticks primarily from infected deer, causes human monocytic ehrlichiosis, an acute febrile systemic illness. This paper reviews current knowledge of clinical and biological aspects of infections caused by E. **chaffeensis**. (C) Elsevier, Paris.

AN 1999:440489 SCISEARCH
GA The Genuine Article (R) Number: 203CJ
TI Clinical and biological aspects of infection caused by Ehrlichia **chaffeensis**
AU Rikihisa Y (Reprint)
CS OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, 1925 COFFEY RD, COLUMBUS, OH 43210 (Reprint)
CYA USA
SO MICROBES AND INFECTION, (APR 1999) Vol. 1, No. 5, pp. 367-376.
Publisher: EDITIONS SCIENTIFIQUES MEDICALES ELSEVIER, 23 RUE LINOIS, 75724 PARIS CEDEX 15, FRANCE.
ISSN: 1286-4579.
DT General Review; Journal
FS LIFE
LA English
REC Reference Count: 50
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 53 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB A gene encoding a 28-M)a protein of Ehrlichia **canis** was cloned, sequenced, and expressed, and a comparative molecular analysis with homologous genes off. **canis**, Cowdria ruminantium, and Ehrlichia **chaffeensis** was performed. The complete gene has an 834-bp open reading frame encoding a protein of 278 amino acids with a predicted molecular mass of 30.5 kDa. An N-terminal signal sequence was Identified, suggesting that the protein undergoes posttranslational

modification to a mature 27.7-kDa protein (P28). The E. **canis** p28 gene has significant nucleic acid and amino acid sequence homologies with the E. **chaffeensis** outer membrane protein-1 (omp-1) gene family, with the Cowdria ruminantium map-1 gene, and with other E. **canis** 28-kDa-protein genes. Southern blotting revealed the presence of at least two additional homologous p28 gene copies in the E. **canis** genome, confirming that p28 is a member of a polymorphic multiple-gene family. Amino acid sequence analysis revealed that E. **canis** P28 has four variable regions, and it shares similar surface-exposed regions, antigenicity, and T-cell motifs with E. **chaffeensis** P28. The p28 genes from seven different E. **canis** isolates were identical, indicating that the gene for this major immunoreactive protein is highly conserved. In addition, reactivity of sera from clinical cases of canine ehrlichiosis with the recombinant P28 demonstrated that the recombinant protein may be a reliable serodiagnostic antigen.

AN 1999:364769 SCISEARCH
GA The Genuine Article (R) Number: 193GD
TI Molecular cloning of the gene for a conserved major immunoreactive 28-kilodalton protein of Ehrlichia **canis**: a potential serodiagnostic antigen
AU McBride J W; Yu X J; Walker D H (Reprint)
CS UNIV TEXAS, MED BRANCH, DEPT PATHOL, 301 UNIV BLVD, GALVESTON, TX 77555 (Reprint); UNIV TEXAS, MED BRANCH, DEPT PATHOL, GALVESTON, TX 77555; UNIV TEXAS, MED BRANCH, WHO COLLABORATING CTR TROP DIS, GALVESTON, TX 77555
CYA USA
SO CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (MAY 1999) Vol. 6, No. 3, pp. 392-399.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 1071-412X.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 32
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 54 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Cowdria ruminantium is the etiologic agent of heartwater, a disease causing major economic loss in ruminants in sub-Saharan Africa and the Caribbean, Development of a serodiagnostic test is essential for determining the carrier status of animals from regions where heartwater is endemic, but most available tests give false-positive reactions with sera against related Erhlichia species. Current approaches rely on molecular methods to define proteins and epitopes that may allow specific diagnosis. Two major antigenic proteins (MAPs), MAP1 and MAP2, have been examined for their use as antigens in the serodiagnosis of heartwater, The objectives of this study were (i) to determine if MAP2 is conserved among five geographically divergent strains of C. ruminantium and (ii) to determine if MAP2 homologs are present in Ehrlichia **canis**, the causative agent of canine ehrlichiosis, and Ehrlichia **chaffeensis**, the organism responsible for human monocytic ehrlichiosis. These two agents are closely related to C. ruminantium. The map2 gene from four strains of C, ruminantium was cloned, sequenced, and compared with the previously reported map2 gene from the Crystal Springs strain. Only 10 nucleic acid differences between the strains were identified, and they translate to only 3 amino acid changes, indicating that MAP2 is highly conserved. Genes encoding MAP2 homologs from E. **canis** and E. **chaffeensis** also were cloned and sequenced. Amino acid analysis of MAP2 homologs of E, **chaffeensis** and E. **canis** with MAP2 of C, ruminantium revealed 83.4 and 84.4% identities, respectively. Further analysis of MAP2 and its homologs revealed that the whole protein lacks specificity for heartwater diagnosis. The development of epitope-specific **assays** using this sequence information may produce diagnostic tests suitable for

C. ruminantium and also other related rickettsiae.
AN 1999:212221 SCISEARCH
GA The Genuine Article (R) Number: 174QU
TI Potential value of major antigenic protein 2 for serological diagnosis of heartwater and related ehrlichial infections
AU Bowie M V (Reprint); Reddy G R; Semu S M; Mahan S M; Barbet A F
CS UNIV FLORIDA, COLL VET MED, DEPT PATHOBIOL, POB 110880, GAINESVILLE, FL 32610 (Reprint); UNIV FLORIDA USAID SADC HEARTWATER RES PROJECT, VET RES LAB, HARARE, ZIMBABWE
CYA USA; ZIMBABWE
SO CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (MAR 1999) Vol. 6, No. 2, pp. 209-215.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 1071-412X.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 25
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 55 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB The major outer membrane proteins (OMPs) of the human granulocytic ehrlichiosis (HGE) agent, with molecular sizes of 44 to 47 kDa, are immunodominant antigens in human infection. Monoclonal antibodies (MAbs) to the OMPs were made by immunizing BALB/c mice with the purified HGE agent and then by fusing spleen cells with myeloma cells. The immunologic specificities of three MAbs (3365, 5C11, and 5D13) were examined with five human HGE agent isolates and one tick isolate. By **Western** blot analysis, all three MAbs recognized the HGE agent but not Ehrlichia **chaffeensis**, Ehrlichia sennetsu, Ehrlichia **canis**, or their host cells. MAb 3365 reacted with a 44-kDa protein in the homologous human isolate but not in the remaining five isolates. The two remaining MAbs recognized proteins with molecular sizes of 44 to 47 kDa in all six isolates, **Western** blot results with the OMP fraction of the six isolates were consistent with results with the whole HGE agent. Immunofluorescent-antibody staining and immunogold labeling,vith these MAbs showed that these antigens were primarily present on the membrane of the HGE agent. MAbs 5C11 and 5D13 recognized the recombinant 44-kDa protein by **Western immunoblot** analysis, but MAb 3365 did not. Passive immunization with MAb 3365 was more effective in protecting mice from HGE agent infection than with MAbs 5C11 and 5D13, These MAbs would be useful for analyzing the role of the major OMP antigens in HGE agent infection and for serodiagnosis.
AN 1998:814308 SCISEARCH
GA The Genuine Article (R) Number: 129YG
TI Characterization of monoclonal antibodies to the 44-kilodalton major outer membrane protein of the human granulocytic ehrlichiosis agent
AU Kim H Y; Rikihisa Y (Reprint)
CS OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, 1925 COFFEY RD, COLUMBUS, OH 43210 (Reprint); OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, COLUMBUS, OH 43210
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (NOV 1998) Vol. 36, No. 11, pp. 3278-3284.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0095-1137.
DT Article; Journal
FS LIFE; CLIN
LA English
REC Reference Count: 18
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 56 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AB A 30-kDa major outer membrane protein of Ehrlichia **canis**, the agent of canine ehrlichiosis, is the major antigen recognized by both naturally and ex:perimentally infected dog sera. The protein cross-reacts with a serum against a recombinant 28-kDa protein (rP28), one of the outer membrane proteins of a gene (omp-1) family of Ehrlichia **chaffeensis**, Two DNA fragments of E. **canis** were amplified by PCR with two primer pairs based on the sequences off. **chaffeensis** omp-1 genes, cloned, and sequenced, Each fragment contained a partial 30-kDa protein gene of E. **canis**, Genomic Southern blot analysis with the partial gene probes revealed the presence of multiple copies of these genes in the E, **canis** genome. Three copies of the entire gene (p30, p30-1, and p30a) were cloned and sequenced from the E. **canis** genomic DNA, The open reading frames of the two copies (p30 and p30-1) were tandemly arranged with an intergenic space. The three copies were similar but not identical and contained a semivariable region and three hypervariable regions in the protein molecules. The following genes homologous to three E, **canis** 30-kDa protein genes and the E, **chaffeensis** omp-1 family were identified in the closely related rickettsiae: wsp from Wolbachia sp,. p44 from the agent of human granulocytic ehrlichiosis, msp-2 and msp-4 from Anaplasma marginale, and map-1 from Cowdria ruminantium. Phylogenetic analysis among the three E. **canis** 30-kDa proteins and the major surface proteins of the rickettsiae revealed that these proteins are divided into four clusters and the two E, **canis** 30-kDa proteins are closely related but that the third 30-kDa protein is not. The p30 gene was expressed as a fusion protein, and the antibody to the recombinant protein (rP30) was raised in a molase. The antibody reacted with rP30 and a 30-kDa protein of purified E, **canis**, Twenty-nine indirect fluorescent antibody (IFA)-positive dog plasma specimens strongly recognized the rP30 off. **canis**, To evaluate whether the rP30 is a suitable antigen for serodiagnosis of canine ehrlichiosis, the immunoreactions between rP30 and the whole purified E. **canis** antigen were compared in the dot **immunoblot assay**, Dot reactions of both antigens with IFA-positive dog plasma specimens were clearly distinguishable by the naked eye from those with IFA-negative plasma specimens. By densitometry with a total of 42 IEA-positive and -negative plasma specimens, both, antigens produced results similar in sensitivity and specificity. These findings suggest that the rP30 antigen provides a simple, consistent, and rapid serodiagnosis for canine ehrlichiosis, Cloning of multigenes encoding the 30-kDa major outer membrane proteins off. **canis** will greatly facilitate understanding pathogenesis and immunologic study of canine ehrlichosis and provide a useful tool for phylogenetic analysis.

AN 1998:640290 SCISEARCH

GA The Genuine Article (R) Number: 111CY

TI Cloning and characterization of multigenes encoding the immunodominant 30-kilodalton major outer membrane proteins of Ehrlichia **canis** and application of the recombinant protein for serodiagnosis

AU Ohashi N; Unver A; Zhi N; Rikihisa Y (Reprint)

CS OHIO STATE UNIV, COLL MED, DEPT VET BIOSCI, 1925 COFFEY RD, COLUMBUS, OH 43210 (Reprint); OHIO STATE UNIV, COLL MED, DEPT VET BIOSCI, COLUMBUS, OH 43210

CYA USA

SO JOURNAL OF CLINICAL MICROBIOLOGY, (SEP 1998) Vol. 36, No. 9, pp. 2671-2680.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0095-1137.

DT Article; Journal

FS LIFE; CLIN

LA English

REC Reference Count: 34

L8 ANSWER 57 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AB Antisera against different Ehrlichiae recognize an immunodominant, cross-reacting similar to 28 kDa surface antigen defined as the MAP1 in Cowdria ruminantium. These antigens are considered valuable in developing serodiagnostic tests and recombinant vaccines for Ehrlichiae infections. To evaluate the relationship in three closely related Ehrlichiae, Ehrlichia **chaffeensis**, Ehrlichia **canis**, and C. ruminantium, the structure of the 28 kDa antigen genes was analyzed. We describe the cloning and characterization of DNA encoding genes homologous to MAP1 from E. **chaffeensis** and E. **canis**. The cloned segment of E. **chaffeensis** contains one expressed and four transcriptionally silent tandemly arranged, nonidentical genes; the E. **canis** locus consists of two nonidentical genes. Comparative analysis of these genes revealed the presence of four conserved regions separated by three highly variable regions. B-cell epitope analysis identified three major cross-reacting epitopes that map to the variable regions. Location of the epitopes at the variable regions and the presence of multigene family with only one expressed copy suggest a mechanism of immune evasion in these Ehrlichiae. (C) 1998 Academic Press.

AN 1998:527722 SCISEARCH

GA The Genuine Article (R) Number: ZY101

TI Molecular characterization of a 28 kDa surface antigen gene family of the tribe Ehrlichiae

AU Reddy G R (Reprint); Sulsona C R; Barbet A F; Mahan S M; Burridge M J; Alleman A R

CS KANSAS STATE UNIV, COLL VET MED, DEPT DIAGNOST MED PATHOBIOL, MANHATTAN, KS 66506 (Reprint); UNIV FLORIDA, COLL VET MED, DEPT PATHOBIOL, GAINESVILLE, FL 32610; UNIV FLORIDA, COLL VET MED, DEPT PHYSIOL SCI, GAINESVILLE, FL 32610; UNIV FLORIDA, USAID, SADC, HEARTWATER RES PROJECT, HARARE, ZIMBABWE

CYA USA; ZIMBABWE

SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (29 JUN 1998) Vol. 247, No. 3, pp. 636-643.
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0006-291X.

DT Article; Journal

FS LIFE

LA English

REC Reference Count: 38
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 58 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AB A 44-kDa major outer membrane protein of the human granulocytic ehrlichiosis (HGE) agent is an immunodominant antigen in human infection. A gene encoding this protein was cloned and sequenced. Southern blot results revealed the existence of multigenes homologous to the P44 gene in the genome of the HGE agent. The recombinant 44-kDa protein (rP44) was expressed by using expression vector pET30a. The reactivity of the affinity-purified rP44 was evaluated by **Western immunoblot** analysis and dot blot **immunoassay**. **Western immunoblot** analysis showed that mouse anti-rP44 serum reacted with 44- to 42-kDa proteins in six different HGE agent strains tested except strain 2, in which three proteins of 42, 40, and 38 kDa were recognized. Eleven HGE patient serum samples, a horse anti-HGE serum, and a horse anti-Ehrlichia equi serum recognized the rP44 protein. This suggests that rP44 is an HGE-E. equi group-specific antigen. Neither human anti-Ehrlichia **chaffeensis** serum nor rabbit anti-Borrelia burgdorferi serum reacted with rp44. Sera from two patients coinfected with the HGE agent and B. burgdorferi reacted positively with rP44 and the HGE agent. Sera from 20 HGE patients with indirect fluorescent-antibody (IFA) titers ranging from 1:20 to 1:2,560 gave distinct positive reactions in a dot **immunoblot assay**. There was a positive

correlation between the color densities of the dot reactions and the IFA titers when greater than 50 ng of recombinant antigen per dot was used. The use of the affinity-purified rP44 protein as antigen would provide a more specific, consistent, and simpler serodiagnosis for HGE than the use of whole infected cells or purified HGE agents.

AN 1998:394296 SCISEARCH
GA The Genuine Article (R) Number: ZN392
TI Cloning and expression of the 44-kilodalton major outer membrane protein gene of the human granulocytic ehrlichiosis agent and application of the recombinant protein to serodiagnosis
AU Zhi N; Ohashi N; Rikihisa Y (Reprint); Horowitz H W; Wormser G P; Hechemy K
CS OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, 1925 COFFEY RD, COLUMBUS, OH 43210 (Reprint); OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, COLUMBUS, OH 43210; NEW YORK MED COLL, WESTCHESTER CTY MED CTR, DIV INFECT DIS, VALHALLA, NY 10595; NEW YORK STATE DEPT HLTH, ALBANY, NY
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (JUN 1998) Vol. 36, No. 6, pp. 1666-1673.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0095-1137.
DT Article; Journal
FS LIFE; CLIN
LA English
REC Reference Count: 31
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 59 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Several immunodominant major proteins ranging from 23 to 30 kDa were identified in the outer membrane fractions of Ehrlichia **chaffeensis** and Ehrlichia **canis**. The N-terminal amino acid sequence of a 28-kDa protein of E. **chaffeensis** (one of the major proteins) was determined, The gene (p28), almost full length, encoding the 28-M)a protein was cloned by PCR with primers designed based on the N-terminal sequence of the E. **chaffeensis** 28-kDa protein and the consensus sequence between the C termini of the Cowdria ruminantium MAP-1 and Anaplasma marginale MSP-4 proteins, The p28 gene was overexpressed, and antibody to the recombinant protein was raised in a rabbit, The antibody and serum from a patient infected with E. **chaffeensis** reacted with the recombinant protein, three proteins (29, 28, and 25 kDa) off. **chaffeensis**, and a 30-kDa protein off. **canis**. Immunoelectron microscopy,vith the rabbit antibody revealed that the antigenic epitope of the 28-kDa protein was exposed on the surface of E. **chaffeensis**. Southern blot analysis with a P-32-labeled p28 gene probe revealed multiple copies of genes homologous to p28 in the E. **chaffeensis** genome. Six copies of the p28 gene were cloned and sequenced from the genomic DNA by using the same probe, The open reading frames of these gene copies were tandemly arranged with intergenic spaces, They were nonidentical genes and contained a semivariable region and three hypervariable regions in the predicted protein molecules, One of the gene copies encoded a protein with an internal amino acid sequence identical to the chemically determined N-terminal amino acid sequence of a 23-kDa protein of E. **chaffeensis**. Immunization with the recombinant P28 protein protected mice from infection with E. **chaffeensis**. These findings suggest that the 30-kDa-range proteins of E. **chaffeensis** represent a family of antigenically related homologous proteins encoded by a single gene family.

AN 1998:52579 SCISEARCH
GA The Genuine Article (R) Number: YP559
TI Immunodominant major outer membrane proteins of Ehrlichia **chaffeensis** are encoded by a polymorphic multigene family
AU Ohashi N; Zhi N; Zhang Y L; Rikihisa Y (Reprint)

CS OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, 1925 COFFEY RD, COLUMBUS, OH 43210 (Reprint); OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, COLUMBUS, OH 43210
CYA USA
SO INFECTION AND IMMUNITY, (JAN 1998) Vol. 66, No. 1, pp. 132-139.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0019-9567.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 44
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 60 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB In order to evaluate the relative sensitivity of the detection of antibodies against various antigenic proteins of Ehrlichia **chaffeensis** for the diagnosis of the emerging infectious disease human monocytotropic ehrlichiosis, **Western** immunoblotting was performed with 27 serum samples from convalescent patients with antibodies, as demonstrated by indirect immunofluorescence **assay** , Among 22 patients with antibodies reactive with the 120-kDa protein, 15 showed reactivity with the 29/28-kDa protein(s) and the proteins in the 44- to 88-kDa range, Two of the serum samples with this pattern reacted with the 29/28-kDa protein(s) of only the 91HE17 strain, and one sample reacted with only that of-the Arkansas strain, indicating that the antibodies were stimulated by strain-specific epitopes. Overall, antibodies to the 29/28-kDa protein(s) were detected in only 16 patients' sera, suggesting that this protein is less sensitive than the 120-kDa protein, Two of 12 serum samples from healthy blood donors had antibodies reactive with the 120-kDa protein; one of these samples reacted also with the 29/28-kDa protein(s) of Ehrlichia **canis**, suggesting that unrecognized ehrlichial infection might have occurred, including human infection with E. **canis**. A high correlation between reactivity with the 120-kDa protein by **Western** immunoblotting and the recombinant 120-kDa protein by dot blot supports the potential usefulness of this recombinant antigen in diagnostic serology.
AN 97:866030 SCISEARCH
GA The Genuine Article (R) Number: YG430
TI **Western** immunoblotting analysis of the antibody responses of patients with human monocytotropic ehrlichiosis to different strains of Ehrlichia **chaffeensis** and Ehrlichia **canis**
AU Chen S M; Cullman L C; Walker D H (Reprint)
CS UNIV TEXAS, MED BRANCH, DEPT PATHOL, 301 UNIV BLVD, GALVESTON, TX 77555 (Reprint); UNIV TEXAS, MED BRANCH, DEPT PATHOL, GALVESTON, TX 77555; MRL DIAGNOST, CYPRESS, CA 90630
CYA USA
SO CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (NOV 1997) Vol. 4, No. 6, pp. 731-735.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 1071-412X.
DT Article; Journal
FS CLIN
LA English
REC Reference Count: 23
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 61 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB The etiologic agent of human granulocytic ehrlichiosis (HGE) is an obligate intracellular bacterium, In 1995, blood specimens from 53 patients suspected of having HGE were examined by indirect fluorescent antibody (IFA) testing with the HGE agent no, 13 isolate as the antigen, by nested PCR, and by culture, All patients resided in Westchester County,

N.Y. Twelve patient specimens were positive for IFA (titer greater than or equal to 1:40), Seven of these were also positive by PCR. Of the seven specimens positive by both IFA testing and PCR, the HGE agent was isolated from four (no, 2, 3, 6, anti II) and continuously cultured in HL-60 cells, These were confirmed as the HGE agent by sequencing of 16S rDNA, Both purified whole-cell organisms and the outer membrane fractions of the new isolates were compared with no, 13 isolate and a tick (USG) isolate by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and **Western immunoblot** analysis, No, 11 and 13 isolates had identical SDS-PAGE patterns with respect to 49- and 47-kDa proteins, Na, 3 and USG isolates lacked the 47-kDa protein, and no, 6 isolate lacked the 49-kDa protein, Both 49- and 47-kDa bands were absent in no, 2 isolate, **Western** blot results with seven different sera, including five convalescent-phase sera from these patients, one dog anti-USG isolate, and one horse anti-BDS isolate, showed that all major antigens in six isolates were recognized by all sera, However, the molecular sizes and the numbers of major antigens recognized varied among the six isolates, Overall, HGE agent no, 3, 6, 11, and 13, and USG isolates had similar patterns, with 1 or 2 major antigens with molecular masses of around 49 and 47 kDa. No, 2 isolate was quite distinct in having a major antigen of 43 kDa. This indicates that although these antigenic epitopes are all cross-reactive among strains, the HGE agent has a strain pleomorphism in its major antigenic proteins. The major antigen profiles of the outer membrane protein fractions and of whole organisms of six HGE agent isolates were similar, suggesting that 49- and 47-kDa major antigens are the outer membrane proteins of the HGE agent.

AN 97:714502 SCISEARCH
GA The Genuine Article (R) Number: XX182
TI Comparison of major antigenic proteins of six strains of the human granulocytic ehrlichiosis agent by **western immunoblot** analysis
AU Zhi N; Rikihisa Y (Reprint); Kim H Y; Wormser G P; Horowitz H W
CS OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, 1925 COFFFEY RD, COLUMBUS, OH 43210 (Reprint); OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, COLUMBUS, OH 43210; NEW YORK MED COLL, WESTCHESTER CTY MED CTR, DIV INFECT DIS, VALHALLA, NY 10595
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (OCT 1997) Vol. 35, No. 10, pp. 2606-2611.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0095-1137.
DT Article; Journal
FS LIFE; CLIN
LA English
REC Reference Count: 26
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 62 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB A polymerase chain reaction (PCR)-based detection **assay** that specifically detected Ehrlichia **canis** in dogs with acute infections was developed. A region of the 16S ribosomal RNA gene of E. **canis** was targeted for PCR amplification and chemiluminescent hybridization (CH) with a complementary internal 287-base pair (bp) oligonucleotide probe. The CH improved the PCR **assay** sensitivity 1,000-fold as compared with visualization on ethidium bromide-stained agarose gels. The PCR **assay** with CH (PCR/CH) detected as little as 30 fg of E. **canis** genomic DNA, the equivalent of approximately 150 E. **canis** organisms. The 495-bp product defined by the specific primers was not detected when genomic DNA from E. platys, E. **chaffeensis**, E. risticii, and E. equi were used in the PCR/CH **assay**. The PCR/CH **assay** was tested with unfractionated blood samples collected from 9 dogs experimentally infected with E. **canis**. The PCR/CH **assay** had greater detection

sensitivity than did cell culture isolation (CCI) from infected blood. PCR/CH detected E. **canis** 7 days prior to CCI in 4 of 6 experimentally infected dogs. The results obtained with the PCR/CH **assay** otherwise consistently matched the results obtained by CCI. This PCR/CH **assay** is a rapid, sensitive, and specific method for E. **canis** detection with sensitivity comparable to or exceeding that of CCI. A diagnosis of E. **canis** using this PCR/CH **assay** can be made in 2 days as compared with 1-4 weeks for CCI. The PCR/CH **assay** appears to be an acceptable alternative or complement to current diagnostic techniques.

AN 97:571511 SCISEARCH
GA The Genuine Article (R) Number: XM719
TI PCR detection of acute Ehrlichia **canis** infection in dogs
AU McBride J W (Reprint); Corstvet R E; Gaunt S D; Chinsangaram J; Akita G Y; Osburn B I
CS UNIV CALIF DAVIS, SCH VET MED, DEPT VET PATHOL MICROBIOL & IMMUNOL, DAVIS, CA 95616 (Reprint); LOUISIANA STATE UNIV, SCH VET MED, DEPT MICROBIOL & PARASITOL, BATON ROUGE, LA 70803; LOUISIANA STATE UNIV, SCH VET MED, DEPT PATHOL, BATON ROUGE, LA 70803
CYA USA
SO JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (OCT 1996) Vol. 8, No. 4, pp. 441-447.
Publisher: AMER ASSOC VETERINARY LABORATORY DIAGNOSTICIANS INC, 1600 E ROLLINS, COLUMBIA, MO 65211.
ISSN: 1040-6387.
DT Article; Journal
FS AGRI
LA English
REC Reference Count: 11
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 63 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Historically, considerable variation has been reported in the type and severity of clinical and hematologic abnormalities associated with canine ehrlichiosis. Because of difficulties associated with the isolation of intracellular monocytic Ehrlichia species in tissue culture systems, few E. **canis** isolates are available for comparative microbiologic studies. To address the issue of potential E. **canis** antigenic diversity in different regions of the world, dog sera reactive by indirect fluorescent antibody testing to E. **canis** (Florida) antigen were obtained from France, Israel, Italy, the United States, the Virgin Islands, and Zimbabwe. Ehrlichia **canis** proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and at least 5 sera from each region were stained by **western** immunoblotting. Antibody immunodominance was scored based upon staining intensity. There was relative homogeneity in the immunogenic protein reactions to E. **canis** antigens. Of the 58 E. **canis** reactive sera, 54 samples resulted in **immunoblot** patterns indicative of chronic ehrlichiosis. Four reactive sera (reciprocal titers of 160-2,560) did not recognize any genus-specific antigens resulting in protein bands between 22 and 29 kD, indicating serologic cross-reactivity with other microorganisms. Relatively homogenous **immunoblot** patterns, consistent with the reported **immunoblot** response of dogs with experimental chronic ehrlichiosis, were observed with sera from Arizona, France, Israel, North Carolina, Texas, and the Virgin Islands. In contrast, unique major proteins were observed in dog sera from Italy and Zimbabwe. Our results indicate that although relatively homogeneous, antigenic diversity may exist among E. **canis** organisms in different regions of the world.

AN 97:571463 SCISEARCH
GA The Genuine Article (R) Number: XM721
TI **Immunoblot** analysis of the immunoglobulin G response to Ehrlichia **canis** in dogs: an international survey
AU Hegarty B C; Levy M G; Gager R F; Breitschwerdt E B (Reprint)

CS N CAROLINA STATE UNIV, COLL VET MED, DEPT COMPAN ANIM & SPECIAL SPECIES MED, RALEIGH, NC 27606 (Reprint); N CAROLINA STATE UNIV, COLL VET MED, DEPT COMPAN ANIM & SPECIAL SPECIES MED, RALEIGH, NC 27606; N CAROLINA STATE UNIV, COLL VET MED, DEPT MICROBIOL PATHOL & PARASITOL, RALEIGH, NC 27606
CYA USA
SO JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (JAN 1997) Vol. 9, No. 1, pp. 32-38.
Publisher: AMER ASSOC VETERINARY LABORATORY DIAGNOSTICIANS INC, 1600 E ROLLINS, COLUMBIA, MO 65211.
ISSN: 1040-6387.
DT Article; Journal
FS AGRI
LA English
REC Reference Count: 22
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 64 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Cowdria ruminantium is the etiologic agent of heartwater, a tick-transmitted foreign animal disease with considerable potential for entrance into the USA. A competitive enzyme-linked immunosorbent **assay** (cELISA) was developed to detect serologic responses to C. ruminantium infection. The cELISA utilized a recombinant form of the C. ruminantium major antigenic protein (MAP-1) as the antigen and an anti-MAP-1 monoclonal antibody as the competing indicator reagent. Experimental antisera to C. ruminantium and a wide variety of related ehrlichial organisms were used to evaluate cELISA reactivity. Only sera against C. ruminantium, Ehrlichia **canis**, E. **chaffeensis**, and a recently discovered cervine ehrlichia-like organism reacted positively in the cELISA. Specificity of the cELISA was greater than or equal to 99.5% in a survey of 1,774 southeastern US and Puerto Rican slaughter cattle sera but was only 85% in a group of 79 hunter-killed white-tailed deer (Odocoileus virginianus) from the southeastern USA. Reference true-positive and cELISA false-positive sera were further analyzed by end point titrations using the cELISA and by indirect fluorescent antibody (IFA) tests for reactivity with C. ruminantium, E. **canis**, and E. **chaffeensis** antigens. True heartwater-positive sera were significantly more reactive using the cELISA and C. ruminantium IFA procedures ($P < 0.05$), whereas false-positive sera were significantly more reactive with the antigens used in the E. **chaffeensis** IFA procedure ($P < 0.05$). A group of sera from 210 field-origin ruminants residing on known or potentially heartwater-endemic Caribbean islands revealed a substantial (12.4%) prevalence of cELISA-positive specimens. The cELISA is a relatively specific serodiagnostic test for heartwater in cattle and could be used to monitor for possible introduction of the disease into the USA. The cELISA may also be an excellent tool for monitoring the success of an ongoing Caribbean Amblyomma tick eradication program designed to eliminate the biological vector responsible for the perpetuation and spread of this dangerous foreign animal disease.
AN 97:571427 SCISEARCH
GA The Genuine Article (R) Number: XM722
TI Development and evaluation of a recombinant antigen, monoclonal antibody-based competitive **ELISA** for heartwater serodiagnosis
AU Katz J B (Reprint); DeWald R; Dawson J E; Camus E; Martinez D; Mondry R
CS ANIM & PLANT HLTH INSPECT SERV, NATL VET SERV LABS, VET SERV, USDA, AMES, IA 50010 (Reprint); CTR DIS CONTROL & PREVENT, US DEPT HHS, ATLANTA, GA 30333; CTR COOPERAT INT RECH AGRON DEV, POINTE A PITRE 97165, GUADELOUPE
CYA USA; GUADELOUPE
SO JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (APR 1997) Vol. 9, No. 2, pp. 130-135.
Publisher: AMER ASSOC VETERINARY LABORATORY DIAGNOSTICIANS INC, 1600 E ROLLINS, COLUMBIA, MO 65211.
ISSN: 1040-6387.

DT Article; Journal
FS AGRI
LA English
REC Reference Count: 20
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 65 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB A partial 16S rRNA gene was amplified in Ehrlichia **canis** -infected cells by nested PCR, The **assay** was specific and did not amplify the closely related Ehrlichia **chaffeensis**, Ehrlichia muris, Neorickettsia helminthoeca, and SF agent 16S rRNA genes, The **assay** was as sensitive as Southern hybridization, detecting as little as 0.2 pg of E. **canis** DNA, By this method, all blood samples from four dogs experimentally infected with E. **canis** were positive as early as day 4 postinoculation, which was before or at the time of seroconversion, One hundred five blood samples from dogs from Arizona and Texas (areas off. **canis** endemicity) and 30 blood samples from dogs from Ohio (area of E. **canis** nonendemicity) were examined by nested PCR and immunofluorescent-antibody (IFA) test. Approximately 84% of dogs from Arizona and Texas had been treated with doxycycline before submission of blood specimens, Among Arizona and Texas specimens, 46 samples were PCR positive (44%) and 80 were IFA positive (76%). Forty-three of 80 IFA-positive samples (54%) were PCR positive, and 22 of 25 IFA-negative samples (88%) mere negative in the nested PCR, None of the Ohio specimens were IFA positive, but 5 specimens were PCR positive (17%), Our results indicate that the nested PCR is highly sensitive and specific for detection of E. **canis** and may be more useful in assessing the clearance of the organisms after antibiotic therapy than IFA, especially in areas in which E. **canis** is endemic.
AN 97:471683 SCISEARCH
GA The Genuine Article (R) Number: XE591
TI Comparison of nested PCR with immunofluorescent-antibody **assay** for detection of Ehrlichia **canis** infection in dogs treated with doxycycline
AU Wen B H; Rikihisa Y (Reprint); Mott J M; Greene R; Kim H Y; Zhi N; Couto G C; Unver A; Bartsch R
CS OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, 1925 COFFEY RD, COLUMBUS, OH 43210 (Reprint); OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, COLUMBUS, OH 43210; OHIO STATE UNIV, DEPT VET CLIN SCI, COLUMBUS, OH 43210; SW VET DIAGNOST CTR, PHOENIX, AZ
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (JUL 1997) Vol. 35, No. 7, pp. 1852-1855.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0095-1137.
DT Article; Journal
FS LIFE; CLIN
LA English
REC Reference Count: 21
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 66 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB The role of white-tailed deer (Odocoileus virginianus) in the epidemiology of Ehrlichia **chaffeensis** and the agent of human granulocytic ehrlichiosis (HGE) is not. fully understood, and diagnostic procedures may be complicated by the recent detection of 16S rDNA sequence from an Ehrlichia sp.-like organism in wild deer. A specific forward primer (DGA) and an Ehrlichia spp, reverse primer (GAIUR) were constructed to amplify this new, distinct Ehrlichia sp.-like 16S rDNA. The DGA primer, a forward primer specific for E. **chaffeensis** (DCH), forward primer specific for the E. phagocytophila genogroup (GE9f) were each used with GA1UR in nested polymerase chain reactions to amplify 16S rDNA sequences from control samples containing the deer Ehrlichia sp.-like

organism, E. **chaffeensis**, or the HGE agent. Primer pairs DGA/GA1UR and DCH/GA1UR specifically amplified 16S rDNA sequences from the corresponding target organism, whereas GE9f/GA1UR amplified 16S rDNA sequence from both tile HGE agent and the deer Ehrlichia sp.-like organism. With a nested PCR using DGA/GA1UR and DCH/GA1UR on DNA extracted from white blood cells from 62 deer from 10 populations in four U.S. states, we observed a high prevalence (65%) of 16S rDNA sequences of the deer Ehrlichia sp.-like organism, and a low prevalence (5%) of the E. **chaffeensis** sequence. In this field survey, E. **chaffeensis** -reactive antibodies detected by indirect fluorescence **assays** were associated ($P < 0.001$) with PCR evidence of the deer Ehrlichia sp.-like organism, but not E. **chaffeensis**. Infestations of Amblyomma americanum also were associated ($P < 0.001$) with PCR evidence of the deer Ehrlichia sp.-like organism. The potential for serologic cross-reactions and non-specific PCR products arising from the deer Ehrlichia sp.-like organism should be considered when evaluating the role of deer and their ticks in the epidemiology of ehrlichial pathogens of humans.

AN 97:349067 SCISEARCH
GA The Genuine Article (R) Number: WW770
TI Development and use of specific polymerase reaction for the detection of an organism resembling Ehrlichia sp. in white-tailed deer
AU Little S E (Reprint); Dawson J E; Lockhart J M; Stallknecht D E; Warner C K; Davidson W R
CS UNIV GEORGIA, COLL VET MED, SE COOPERAT WILDLIFE DIS STUDY, ATHENS, GA 30602 (Reprint); US DEPT HHS, VIRAL & RICKETTSIAL ZOONOSES BRANCH, DIV VIRAL & RICKETTSIAL DIS, ATLANTA, GA 30333; UNIV GEORGIA, DB WARNELL SCH FOREST RESOURCES, ATHENS, GA 30602
CYA USA
SO JOURNAL OF WILDLIFE DISEASES, (APR 1997) Vol. 33, No. 2, pp. 246-253. Publisher: WILDLIFE DISEASE ASSN, INC, 810 EAST 10TH ST, LAWRENCE, KS 66044-8897.
ISSN: 0090-3558.
DT Article; Journal
FS AGRI
LA English
REC Reference Count: 23
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 67 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB DNA encoding two repeat units of the 120-kDa protein of Ehrlichia **chaffeensis** was cloned into the expression vector pGEX and expressed in Escherichia coli. The sensitivity and specificity of a dot blot **assay** for detection of human antibodies with the recombinant protein were 86 and 100%, respectively, compared with an indirect immunofluorescence **assay**.
AN 96:768876 SCISEARCH
GA The Genuine Article (R) Number: VM556
TI THE RECOMBINANT 120-KILODALTON PROTEIN OF EHRLICHIA-**CHAFFEENSIS**, A POTENTIAL DIAGNOSTIC-TOOL
AU YU X J; CROCQUETVALDES P; CULLMAN L C; WALKER D H (Reprint)
CS UNIV TEXAS, MED BRANCH, DEPT PATHOL, 301 UNIV BLVD, GALVESTON, TX, 77555 (Reprint); UNIV TEXAS, MED BRANCH, DEPT PATHOL, GALVESTON, TX, 77555; MRL DIAGNOST, CYPRESS, CA, 90630
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (NOV 1996) Vol. 34, No. 11, pp. 2853-2855.
ISSN: 0095-1137.
DT Article; Journal
FS LIFE; CLIN
LA ENGLISH
REC Reference Count: 10
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 68 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AB We report the first isolation and molecular and antigenic characterization of a human ehrlichial species in South America. A retrospective study was performed with serum specimens from 6 children with clinical signs suggestive of human ehrlichiosis and 43 apparently healthy adults who had a close contact with dogs exhibiting clinical signs compatible with canine ehrlichiosis. The evaluation was performed by the indirect fluorescent-antibody **assay** with Ehrlichia **chaffeensis** Arkansas, Ehrlichia **canis** Oklahoma, and Ehrlichia muris antigens. The sera from two apparently healthy humans were positive by the indirect fluorescent-antibody **assay** for all three antigens. Of the three antigens, samples from humans 1 and 2 showed the highest antibody titers against E. **chaffeensis** and E. muris, respectively. The remaining serum samples were negative for all three antigens. One year later examination of a blood sample from subject 1 revealed morulae morphologically resembling either E. **canis**, E. **chaffeensis**, or E. muris in monocytes in the blood smear. The microorganism, referred to here as Venezuelan human ehrlichia (VHE), was isolated from the blood of this person at 4 days after coculturing isolated blood leukocytes with a dog macrophage cell line (DH82). The organism was also isolated from mice 10 days after intraperitoneal inoculation of blood leukocytes from subject 1. Analysis by electron microscopy showed that the human isolate was ultrastructurally similar to E. **canis**, E. **chaffeensis**, and E. muris. When the virulence of VHE in mice was compared with those of E. **chaffeensis** , E. **canis**, and E. muris, only VHE and E. muris induced clinical signs in BALB/c mice at 4 and 10 days, respectively, after intraperitoneal inoculation. VHE was reisolated from peritoneal exudate cells of the mice. Only E. **chaffeensis**- and E. muris-infected mice developed significant splenomegaly. **Western immunoblot** analysis showed that serum from subject 1 reacted with major proteins of the VHE antigen of 110, 80, 76, 58, 43, 35, and 34 kDa. Human serum against E. **chaffeensis** reacted strongly with 58-, 54-, 52-, and 40-kDa proteins of the VHE antigen. Anti-E. **canis** dog serum reacted strongly with 26- and 24-kDa proteins of VHE. In contrast, anti-E. sennetsu rabbit and anti-E. muris mouse sera did not react with the VILE antigen. Serum from subject 1 reacted with major proteins of 90, 64, or 47 kDa of the E. **chaffeensis**, E. **canis**, and E. muris antigens. This reaction pattern suggests that this serum sample was similar to serum samples from E. **chaffeensis**-infected human patients in Oklahoma. The base sequence of the 168 rRNA gene of VHE was most closely related to that of E. **canis** Oklahoma. On the basis of these observations, we suggest that VHE is a new strain or a subspecies of E. **canis** which may cause asymptomatic persistent infection in humans.

AN 96:637784 SCISEARCH

GA The Genuine Article (R) Number: VD335

TI EHRLICHIA **CANIS**-LIKE AGENT ISOLATED FROM A MAN IN VENEZUELA - ANTIGENIC AND GENETIC-CHARACTERIZATION

AU PEREZ M; RIKIHISA Y (Reprint); WEN B H

CS OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, COLUMBUS, OH, 43210 (Reprint); OHIO STATE UNIV, COLL VET MED, DEPT VET BIOSCI, COLUMBUS, OH, 43210

CYA USA

SO JOURNAL OF CLINICAL MICROBIOLOGY, (SEP 1996) Vol. 34, No. 9, pp. 2133-2139.
ISSN: 0095-1137.

DT Article; Journal

FS LIFE; CLIN

LA ENGLISH

REC Reference Count: 33
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 69 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AB Objective-To ascertain whether dogs are naturally infected with Ehrlichia **chaffeensis**.

Animals-74 dogs from 5 animal shelters and 1 kennel in 3 cities and 3 counties in southeastern Virginia were tested during June 1991.

Procedure-Blood was drawn from 74 dogs; 73 were tested serologically for antibodies reactive to E **chaffeensis** and E **canis**, and 38 were tested for the presence of E **chaffeensis**, E **canis**, and E ewingii by polymerase chain reaction (PCR). Serologic testing by indirect fluorescent antibody **assay**. Nested PCR used Ehrlichia-wide outside primers to detect initial products, followed by use of species-specific primers for identification.

Results-28 (38.4%) dogs had a positive test result (minimum titer, greater than or equal to 1:64) for antibodies reactive to E **chaffeensis**, and 28 (38.4%) had a positive reaction to E **canis**. PCR analysis indicated that 8 (42.1%) dogs were positive for E **chaffeensis** and 6 dogs (31.6%) were positive for E ewingii All dogs had negative results of the PCR test for E **canis**.

Conclusion-Dogs are potential reservoirs of E **chaffeensis**.

Clinical Relevance-Canine E **chaffeensis** infection may be more prevalent than E **canis** or E ewingii infection in this region of the United States.

AN 96:590323 SCISEARCH

GA The Genuine Article (R) Number: VA590

TI POLYMERASE CHAIN-REACTION EVIDENCE OF EHRLICHIA-**CHAFFEENSIS**, AN ETIOLOGIC AGENT OF HUMAN EHRLICHIOSIS, IN DOGS FROM SOUTHEAST VIRGINIA

AU DAWSON J E (Reprint); BIGGIE K L; WARNER C K; COOKSON K; JENKINS S; LEVINE J F; OLSON J G

CS CTR DIS CONTROL & PREVENT, VIRAL & RICKETTSIAL ZOONOSES BRANCH, DIV VIRAL & RICKETTSIAL DIS, ATLANTA, GA, 30333 (Reprint); N CAROLINA STATE UNIV, DEPT MICROBIOL PATHOL & PARASITOL, RALEIGH, NC, 27606; VIRGINIA DEPT HLTH, RICHMOND, VA, 23219

CYA USA

SO AMERICAN JOURNAL OF VETERINARY RESEARCH, (AUG 1996) Vol. 57, No. 8, pp. 1175-1179.
ISSN: 0002-9645.

DT Article; Journal

FS AGRI

LA ENGLISH

REC Reference Count: 18

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 70 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AB Recombinant baculovirus techniques were used to express the 260 amino acid carboxyterminal portion of the 32 kilodalton (kDa) major antigenic protein (MAP 1) of Cowdria ruminantium, the heartwater agent, as a fusion protein. The recombinant MAP 1 was fused to an aminoterminal independently antigenic octapeptide sequence (FLAG(R) peptide). Recombinant MAP 1 was used as an immunoblotting antigen to evaluate numerous reference antisera against organisms of the tribe Ehrlichieae. Monoclonal and polyclonal C. ruminantium antibodies, monoclonal anti-FLAG(R) ascites, and antisera to Ehrlichia **canis** and Ehrlichia **chaffeensis** reacted with this antigen. Twelve of 79 sera collected 1980 to 1992 from southeastern U.S. white-tailed deer (Odocoileus virginianus) were also unexpectedly **immunoblot**-positive to MAP 1. These 12 deer sera had, as a group, significantly ($P < 0.01$) greater anti-E. **chaffeensis** titers (previously determined) than the sera from MAP 1 **immunoblot** -negative deer living in the same areas. None of the 262 sera from cattle living in tile same areas were **immunoblot**-positive to MAP 1. All of an additional 50 cervine sera from Michigan (USA), 72 bovine sera from northern U.S. cattle, and 72 sera from Puerto Rican cattle were also **immunoblot**-negative to MAP 1. Sera from African sheep which were falsely seropositive to authentic MAP 1 were also **immunoblot** -positive to the recombinant MAP 1. Unidentified Ehrlichia spp, capable of serologic crossreactivity with the heartwater agent appear to be present

in some southeastern U.S. white-tailed deer but not cattle. These or related Ehrlichia spp. may also be found elsewhere in the world in non-cervine species.

AN 96:544165 SCISEARCH
GA The Genuine Article (R) Number: UX347
TI A RECOMBINANT ANTIGEN FROM THE HEARTWATER AGENT (COWDRIA-RUMINATIUM) REACTIVE WITH ANTIBODIES IN SOME SOUTHEASTERN UNITED-STATES WHITE-TAILED DEER (ODOCOILEUS-VIRGINIANUS), BUT NOT CATTLE, SERA
AU KATZ J B (Reprint); BARBET A F; MAHAN S M; KUMBULA D; LOCKHART J M; KEEL M K; DAWSON J E; OLSON J G; EWING S A
CS US ANIM & PLANT HLTH INSPECT SERV, USDA, VET SERV, NATL VET SERV LABS, DIAGNOST VIROL LAB, AMES, IA, 50010 (Reprint); UNIV FLORIDA, DEPT INFECT DIS, GAINESVILLE, FL, 32611; HEARTWATER RES PROJECT, VET RES LAB, HARARE, ZIMBABWE; UNIV GEORGIA, COLL VET MED, SE COOPERAT WILDLIFE DIS STUDY, ATHENS, GA, 30062; OKLAHOMA STATE UNIV, COLL VET MED, STILLWATER, OK, 74078; CTR DIS CONTROL & PREVENT, NATL CTR INFECT DIS, US PHS, ATLANTA, GA, 30333
CYA USA; ZIMBABWE
SO JOURNAL OF WILDLIFE DISEASES, (JUL 1996) Vol. 32, No. 3, pp. 424-430. ISSN: 0090-3558.
DT Article; Journal
FS AGRI
LA ENGLISH
REC Reference Count: 25
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 71 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Ehrlichia **chaffeensis**, an obligately intracellular bacterium with tropism for monocytes, is the etiologic agent of human monocytic ehrlichiosis. To determine the nature and ultrastructural location of E. **chaffeensis** antigens, monoclonal antibodies (MAbs) to E. **chaffeensis** were developed. The MAbs were used for immunofluorescence and **Western** immunoblotting analysis of the antigens of density gradient-purified ehrlichiae. Monoclonal antibody 6A1 recognized an epitope of a 30-kD protein. This antibody reacted with a strain-specific epitope of E. **chaffeensis**, Arkansas strain, and did not cross-react with any other ehrlichia tested. Monoclonal antibodies 3C7 and 7C1-B recognized Arkansas strain proteins of 30 and 29 kD and reacted with E, **chaffeensis** (strain 91HE17) proteins of 31 and 29 kD and an E. **canis** protein of 30 kD. Lack of reactivity of these two MAbs with E. sennetsu and E. risticii suggests that the epitope is group-specific. Monoclonal antibody 5D11 recognized a 58-kD protein of both strains of E. **chaffeensis** as well as E. **canis**, apparently a group-specific, conformation-independent epitope. Monoclonal antibody 7C1-C reacted with 58- and 88-kD proteins of both the Arkansas and 91HE17 strains. Trypsin treatment destroyed the reactivity of E. **chaffeensis** antigens with all the MAbs when tested by **Western** immunoblotting, indicating that these antigens are proteins with trypsin-sensitive epitopes. Immunoelectron microscopy of negatively stained intact E. **chaffeensis** organisms showed that the 30- and 29-kD proteins are present on the surface of the ehrlichial cell wall along with the previously localized 28-kD protein.

AN 96:364954 SCISEARCH
GA The Genuine Article (R) Number: UJ490
TI ANALYSIS AND ULTRASTRUCTURAL-LOCALIZATION OF EHRLICHIA-**CHAFFEENSIS** PROTEINS WITH MONOCLONAL-ANTIBODIES
AU CHEN S M (Reprint); POPOV V L; FENG H M; WALKER D H
CS UNIV TEXAS, MED BRANCH, DEPT PATHOL, GALVESTON, TX, 77555 (Reprint)
CYA USA
SO AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE, (APR 1996) Vol. 54, No. 4, pp. 405-412. ISSN: 0002-9637.
DT Article; Journal
FS LIFE; CLIN

LA ENGLISH
REC Reference Count: 25
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 72 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Currently available serological tests for cowdriosis (Cowdria ruminantium infection) in domestic ruminants are hampered by their low specificities because of cross-reactivity with Ehrlichia spp, The use of recombinant major antigenic protein (MAP1) of C. ruminantium for serodiagnosis was investigated, Overlapping fragments of the MAP1 protein were expressed in Escherichia coli and were reacted with sera from sheep infected with either C. ruminantium or Ehrlichia ovina, Two immunogenic regions on the MAP1 protein, designated MAP1-A and MAP1-B, were identified. MAP1-A was reactive with C. ruminantium antisera, E. ovina antisera, and three MAP1-specific monoclonal antibodies, whereas MAP1-B reacted only with C. ruminantium antisera, An indirect enzyme-linked immunosorbent **assay** (**ELISA**) based on MI-B was further developed and validated with sera from animals experimentally infected with C. ruminantium or several Ehrlichia spp, Antibodies raised in sheep, tattle, and goats against nine isolates of C. ruminantium reacted with MAP1-B. Cross-reactivity with MAP1-B was limited to Ehrlichia **canis** and Ehrlichia **chaffeensis**, two rickettsias which do not infect ruminants. Antibodies to Ehrlichia spp, which do infect ruminants (E. bovis, E. ovina, and E. phagocytophila) did not react with MAP1-B. Antibody titers to C. ruminantium in sera from experimentally infected cattle, goats, and sheep were detectable for 50 to 200 days postinfection. Further validation of the recombinant MAP1-B-based **ELISA** was done with sera obtained from sheep raised in heartwater-free areas in Zimbabwe and from several Caribbean islands. A total of 159 of 169 samples which were considered to be false positive by immunoblotting or indirect **ELISA** did not react with MAP1-B, In conclusion, recombinant MAP1-B may be a suitable antigen for a sensitive serological test for cowdriosis, with dramatically improved specificity.
AN 95:564224 SCISEARCH
GA The Genuine Article (R) Number: RP755
TI USE OF A SPECIFIC IMMUNOGENIC REGION ON THE COWDRIA-RUMINANTIUM MAP1 PROTEIN IN A SEROLOGICAL **ASSAY**
AU VANVLIET A H M; VANDERZEIJST B A M; CAMUS E; MAHAN S M; MARTINEZ D; JONGEJAN F (Reprint)
CS UNIV UTRECHT, FAC VET MED, INST INFECT DIS & IMMUNOL, DEPT BACTERIOL, POB 80165, 3508 TD UTRECHT, NETHERLANDS (Reprint); UNIV UTRECHT, FAC VET MED, INST INFECT DIS & IMMUNOL, DEPT BACTERIOL, 3508 TD UTRECHT, NETHERLANDS; CTR COOPERAT INT RECH AGRON DEV, DEPT ELEVAGE & MED VET, POINTE A PITRE 97185, GUADELOUPE; UNIV FLORIDA, US AGCY INT DEV, SADC, HEARTWATER RES PROJECT, HARARE, ZIMBABWE
CYA NETHERLANDS; GUADELOUPE; ZIMBABWE
SO JOURNAL OF CLINICAL MICROBIOLOGY, (SEP 1995) Vol. 33, No. 9, pp. 2405-2410.
ISSN: 0095-1137.
DT Article; Journal
FS LIFE; CLIN
LA ENGLISH
REC Reference Count: 35
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 73 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Ehrlichia **chaffeensis** is the causative agent of human monocytic ehrlichiosis, a disease that ranges in severity from asymptomatic infection to death, Only one isolate off. **chaffeensis** has been made, the Arkansas strain, upon which all characterizations of the agent of human monocytic ehrlichiosis have been based, We report the isolation and characterization of a new strain of E. **chaffeensis**, the 91HE17 strain, which was cultivated from a patient with a nearly fatal illness, The new isolate grows best in culture with careful control

of pH, The two isolates are nearly identical as determined by light and electron microscopy and have significant antigenic identity in fluorescent-antibody and **immunoblot assays** using polyclonal antisera and the E, **chaffeensis**-specific monoclonal antibody 1A9, Isolate 91HE17 had 99.9% nucleotide sequence identity with the Arkansas strain in the 16S rRNA gene, Parts of the Escherichia coli GroE operon homologs had identical restriction enzyme digestion patterns, and a 425-bp region of the GroEL gene had at least 99.8% sequence identity between the E. **chaffeensis** Arkansas and 91HE17 strains. Isolate 91HE17 lacked an epitope identified in E, **chaffeensis** Arkansas by the monoclonal antibody 6A1, This new E, **chaffeensis** isolate is very similar to the Arkansas strain and provides the opportunity to substantiate the existence of diversity among ehrlichiae which infect humans, Specific factors which differ among strains may then be compared to assess their potential contributions toward cellular pathogenicity and ultimately toward the development of disease in humans.

AN 95:432527 SCISEARCH

GA The Genuine Article (R) Number: RD990

TI ISOLATION AND CHARACTERIZATION OF A NEW STRAIN OF EHRLICHIA-**CHAFFEENSIS** FROM A PATIENT WITH NEARLY FATAL MONOCYTIC EHRLICHIOSIS

AU DUMLER J S (Reprint); CHEN S M; ASANOVICH K; TRIGIANI E; POPOV V L; WALKER D H

CS UNIV MARYLAND, MED CTR, DEPT PATHOL, BALTIMORE, MD, 21201 (Reprint); UNIV TEXAS, MED BRANCH, DEPT PATHOL, GALVESTON, TX, 77555

CYA USA

SO JOURNAL OF CLINICAL MICROBIOLOGY, (JUL 1995) Vol. 33, No. 7, pp. 1704-1711.
ISSN: 0095-1137.

DT Article; Journal

FS LIFE; CLIN

LA ENGLISH

REC Reference Count: 30
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 74 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AB Homology in the 16S rDNAs shows that the agent of human granulocytic ehrlichiosis (HGE) is closely related to the veterinary pathogens Erlichia equi and Erlichia phagocytophila. After HGE, patients develop antibodies reactive with E. equi and E. phagocytophila; thus, we hypothesized that these species are closely related and share significant antigenicity. Antisera from humans, horses, dogs, and cattle were tested by indirect fluorescent-antibody **assay** (IFA) for antibodies reactive with E. equi and other ehrlichiae and tested by **immunoblot** to identify the specific reactions with E. equi. All convalescent-phase sera from human patients with HGE and from animals infected or immunized dth E. equi or E. phagocytophila had antibodies reactive with E. equi by IFA; no reactions with Ehrlichia **chaffeensis** occurred with these sera, and only one horse naturally infected with E. equi had a serologic reaction against Ehrlichia sennetsu. Human and animal sera obtained after infection or immunization with other Ehrlichia, Rickettsia, and Bartonella species did not react with E. equi by IFA. E. equi **immunoblots** revealed as many as 19 bands with equine anti-E. equi serum. All HGE agent, E. equi, and E. phagocytophila antisera tested reacted with a 44-kDa antigen of E. equi, while other anti-Ehrlichia spp. sera reacted with this antigen rarely or not at all. HGE agent, E. equi, and E. phagocytophila antisera but not other sera also reacted occasionally with 25-, 42-, and 100-MDa antigens. Most sera reacted with antigens between approximately 56 and 75 kDa, probably heat shock proteins. The HGE agent, E. equi, and E. phagocytophila share significant antigenicity by IPA and **immunoblot**. Coupled with the nearly identical nucleotide sequences of 16S rRNA genes, these data indicate that E. equi, E. phagocytophila, and the human granulocytic ehrlichia are closely related or identical species.

AN 95:281922 SCISEARCH
GA The Genuine Article (R) Number: QT306
TI SEROLOGIC CROSS-REACTIONS AMONG EHRLICHIA-EQUI, EHRLICHIA-PHAGOCYTOPHILA, AND HUMAN GRANULOCYTIC EHRLICHIA
AU DUMLER J S (Reprint); ASANOVICH K M; BAKKEN J S; RICHTER P; KIMSEY R; MADIGAN J E
CS UNIV MARYLAND, MED CTR, DEPT PATHOL, BALTIMORE, MD, 21201 (Reprint); DULUTH CLIN, INFECT DIS SECT, DULUTH, MN, 55805; UNIV CALIF DAVIS, SCH VET MED, DEPT MED & EPIDEMIOL, DAVIS, CA, 95616; UNIV CALIF DAVIS, SCH VET MED, DEPT ENTOMOL, DAVIS, CA, 95616
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (MAY 1995) Vol. 33, No. 5, pp. 1098-1103.
ISSN: 0095-1137.
DT Article; Journal
FS LIFE; CLIN
LA ENGLISH
REC Reference Count: 25
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 75 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Human monocytic ehrlichiosis is caused by Ehrlichia **chaffeensis**, an intracellular bacterium probably transmitted by the tick Amblyomma americanum in the United States. Despite its lack of specificity in discriminating among infections by closely related Ehrlichia spp., immunofluorescence **assay** (IFA) is the most frequently used serological diagnostic method. To improve the specificity of the serological diagnosis, we compared antigenic profile of E. **canis** and E. **chaffeensis** antigen with homologous and heterologous sera, searching for the specificity of the presence of low-molecular-weight proteins. **Western immunoblot** analysis of IFA-positive human sera revealed 27- and 29-kDa proteins which are not found in E. **canis** IFA-positive sera from dogs. IFA-positive sera from dogs revealed a low-molecular-weight group of proteins (20 to 28 kDa) which were not found in human E. **chaffeensis**-positive sera except for a weak band at 22 kDa. The presence of antibodies directed against the 27- and 29-kDa proteins on **Western** blots is specific for E. **chaffeensis** infection, and we suggest that the **Western** blot might complete IFA in cases with low positive predictive value.
AN 94:769271 SCISEARCH
GA The Genuine Article (R) Number: PV209
TI SEROLOGIC DIAGNOSIS OF HUMAN MONOCYTIC EHRLICHIOSIS BY **IMMUNOBLOT** ANALYSIS
AU BROUQUI P (Reprint); LECAM C; OLSON J; RAOULT D
CS FAC MED MARSEILLE, UNITE RICKETTSIES, 27 BLVD J MOULIN, F-13385 MARSEILLE 5, FRANCE (Reprint); CDC ATLANTA, VIRAL & RICKETTSIAL ZOONOSES BRANCH, ATLANTA, GA, 30333
CYA FRANCE; USA
SO CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (NOV 1994) Vol. 1, No. 6, pp. 645-649.
ISSN: 1071-412X.
DT Article; Journal
FS CLIN
LA ENGLISH
REC Reference Count: 28
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 76 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Ehrlichia **chaffeensis**, E. **canis**, and E. ewingii are genetically closely related, as determined by 16S rRNA gene base sequence comparison, but they exhibit biologic differences. E. **chaffeensis** is the etiologic agent of human ehrlichiosis. E. **canis** and E. ewingii cause two distinctly different forms of canine ehrlichiosis and

infect different types of leukocytes, monocytes and granulocytes, respectively. E. **chaffeensis** can also infect dogs. In the study, **Western immunoblot** analysis of sera from dogs inoculated with E. **chaffeensis**, E. **canis**, or E. ewingii was performed to determine antigenic specificity and the intensities of the reactions to purified E. **chaffeensis** and E. **canis** antigens. At 2 to 3 weeks postexposure, antisera from four dogs inoculated with E. **chaffeensis** reacted with 64-, 47-, 31-, and 29-kDa proteins of E. **chaffeensis** but reacted poorly with E. **canis** antigen. In contrast, at 2 to 3 weeks postexposure, antisera from four E. **canis**-inoculated dogs reacted strongly with the 30-kDa major antigen of E. **canis** but reacted poorly with proteins from E. **chaffeensis**. At 4 weeks postexposure, the sera from three E. ewingii-inoculated dogs showed weak binding to 64- and 47-kDa proteins of both E. **chaffeensis** and E. **canis**. Convalescent-phase sera from human ehrlichiosis patients and sera from dogs chronically infected with E. ewingii strongly reacted with similar sets of proteins of E. **chaffeensis** and E. **canis** with similar intensities. However, sera from dogs chronically infected with E. **canis** reacted more strongly with a greater number of E. **canis** proteins than with E. **chaffeensis** proteins. The protein specificity described in the report suggests that dogs with E. **canis** infections can be distinguished from E. **chaffeensis** -infected animals by **Western immunoblot** analysis with both E. **canis** and E. **chaffeensis** antigens.

AN 94:511476 SCISEARCH
GA The Genuine Article (R) Number: PB541
TI **WESTERN IMMUNOBLOT** ANALYSIS OF EHRLICHIA-**CHAFFEENSIS**, EHRLICHIA-**CANIS**, OR E-EWINGII INFECTIONS IN DOGS AND HUMANS
AU RIKIHISA Y (Reprint); EWING S A; FOX J C
CS OHIO STATE UNIV, COLL VET MED, DEPT VET PATHOBIOL, 1925 COFFEY RD, COLUMBUS, OH, 43210 (Reprint); OKLAHOMA STATE UNIV, COLL VET MED, DEPT VET PARASITOL MICROBIOL & PUBL HLTH, STILLWATER, OK, 74078
CYA USA
SO JOURNAL OF CLINICAL MICROBIOLOGY, (SEP 1994) Vol. 32, No. 9, pp. 2107-2112.
ISSN: 0095-1137.
DT Article; Journal
FS LIFE; CLIN
LA ENGLISH
REC Reference Count: 18
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 77 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Objective.-To characterize the clinical presentation and course, laboratory findings, and treatment outcome of 12 patients with human granulocytic ehrlichiosis.

Setting.-The 12 patients were male, ranged in age from 29 to 91 years, and contracted their illness in Wisconsin or Minnesota.

Methods.-Cases were recognized by the presence of intracytoplasmic inclusions (morulae) in peripheral neutrophils of patients presenting with temperature of 38.5 degrees C or higher, chills, severe headache, and myalgias. All patients had a complete blood cell count and blood chemistry profile. Blood smears were examined by light microscopy. All available paired serum samples were analyzed for presence of indirect fluorescent antibodies against Ehrlichia **chaffeensis**, Ehrlichia phagocytophila, and Ehrlichia equi Blood samples from 12 patients were subjected to polymerase chain reaction analysis using primers specific for the E phagocytophilal E equi group, primers that include the agent identified in our patients, as well as E **chaffeensis**.

Results.-Varying combinations of leukopenia, anemia, and thrombocytopenia were found in all but one patient. All 12 patients demonstrated morulae in the cytoplasm of neutrophils, but not in

mononuclear white blood cells. Serum **assays** failed to detect antibodies against E **chaffeensis**, but eight of 10 patients and seven of 10 patients tested had antibody titers of 1:80 or more for E phagocytophila and E equi, respectively. Polymerase chain reaction products obtained with primers for E phagocytophila, E equi, and the granulocytotropic Ehrlichia revealed that seven patients were infected with the same agent. The results of serological **assays** or polymerase chain reaction strongly suggest that all 12 patients were infected by E phagocytophila, E equi, or a closely related Ehrlichia species. Two of the 12 patients died. The other 10 patients improved rapidly with oral doxycycline treatment.

Conclusions.-We believe that all 12 patients have been infected with a granulocytic Ehrlichia species, reflecting a recently described new disease entity. The infective organism appears to be closely related to E phagocytophila and E equi. The geographic domain of human granulocytic ehrlichiosis is currently unknown. This novel granulocytic Ehrlichia species is capable of causing fatal infections in humans. Early detection and treatment with tetracycline drugs appear to offer the best chance for complete recovery.

AN 94:411855 SCISEARCH
GA The Genuine Article (R) Number: NW185
TI HUMAN GRANULOCYTIC EHRLICHIOSIS IN THE UPPER MIDWEST UNITED-STATES - A NEW SPECIES EMERGING
AU BAKKEN J S (Reprint); DUMLER J S; CHEN S M; ECKMAN M R; VANETTA L L; WALKER D H
CS DULUTH CLIN LTD, INFECT DIS SECT, 400 E 3RD ST, DULUTH, MN, 55805 (Reprint); UNIV MARYLAND, DEPT PATHOL, BALTIMORE, MD, 21201; UNIV TEXAS, MED BRANCH, DEPT PATHOL, GALVESTON, TX, 77550
CYA USA
SO JAMA-JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION, (20 JUL 1994) Vol. 272, No. 3, pp. 212-218.
ISSN: 0098-7484.
DT Article; Journal
FS LIFE; CLIN
LA ENGLISH
REC Reference Count: 35
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 78 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Ehrlichia **chaffeensis**, the novel etiologic agent of human ehrlichiosis in the United States, was first isolated in 1990 and reported in 1991. To analyze the antigenic components off. **chaffeensis**, we cultivated these obligate intracellular bacteria in DH82 cells, purified the ehrlichiae by renografin density gradient centrifugation, and examined the antigens by **Western** immunoblotting. Rabbit and human antisera to E. **chaffeensis** revealed more than 20 bands ranging from 20 to 200 kD. The distinct 22-kD protein was heat labile. The rest of the major immunoreactive components were heat stable. The **immunoblots** off. **chaffeensis** were highly similar when probed with antisera to E. **chaffeensis**, E. **canis**, and E. ewingii, indicating the close antigenic relationships among the three species. The 22-kD protein cross-reacted only with anti-E. **canis** serum. The antibody against E. sennetsu reacted strongly with the 66-, 64-, 55-, and 44-kD antigens of E. **chaffeensis**. The E. risticii antisera reacted strongly with the 55- and 44-kB bands but only faintly with the 66-kD band. The major immunoreactive antigens of E. **chaffeensis** (66, 55, and 44 kD) showed cross-reactions with all the different antisera tested. The results indicated that E. **chaffeensis** is antigenically most closely related to E. **canis**, is less closely related to E. ewingii, and is only distantly related to E. sennetsu and E. risticii.
AN 94:132427 SCISEARCH
GA The Genuine Article (R) Number: MW296
TI IDENTIFICATION OF THE ANTIGENIC CONSTITUENTS OF EHRLICHIA-

CHAFFEENSIS
AU CHEN S M (Reprint); DUMLER J S; FENG H M; WALKER D H
CS UNIV TEXAS, MED BRANCH, DEPT PATHOL, GALVESTON, TX, 77550 (Reprint); UNIV MARYLAND, SCH MED, DEPT PATHOL, BALTIMORE, MD, 21201
CYA USA
SO AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE, (JAN 1994) Vol. 50, No. 1, pp. 52-58.
ISSN: 0002-9637.
DT Article; Journal
FS LIFE; CLIN
LA ENGLISH
REC Reference Count: 24
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 79 OF 85 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB An infectious agent was isolated from the enlarged spleen of a wild mouse, Eothenomys kageus, by intraperitoneal inoculation of the spleen homogenate into laboratory mice. The laboratory mice developed splenomegaly, and the agent was maintained by serial passage of spleen homogenates in laboratory mice. The agent in the spleen homogenate was inactivated after incubation at 37 or 50-degrees-C. Tetracyclines were effective in preventing infection of mice with this agent, but penicillin and sulfonamides were ineffective. Cytoplasmic inclusion bodies were observed in the peritoneal macrophages of infected mice. Electron microscopy revealed numerous small pleomorphic cocci within membrane-lined vacuoles in the cytoplasm of splenic macrophages. Morphologically similar to the ehrlichial organisms, each organism was surrounded by a distinct plasma membrane and rippled outer cell membrane without a distinct peptidoglycan layer. The agent did not grow in chicken embryos, and the Weil-Felix test result was negative. In the indirect fluorescent-antibody test, the agent reciprocally cross-reacted with Ehrlichia **canis** and cross-reacted somewhat with Ehrlichia sennetsu but did not cross-react with Ehrlichia risticii, Neorickettsia helminthoeca, Rickettsia tsutsugamushi, or Chlamydia spp. The mouse antiserum against this agent reacted with 64-, 47-, 46-, 44-, and 40-kDa proteins of E. **canis** by **Western** blotting (immunoblotting). Since E. **canis** and closely related Ehrlichia **chaffeensis** and Ehrlichia ewingii are not known to proliferate or cause splenomegaly in mice, these results suggest that the agent is a new species within the tribe Ehrlichieae of the family Rickettsiaceae. The finding suggests that wild rodents may serve as reservoirs for pathogenic ehrlichiae.
AN 93:1806 SCISEARCH
GA The Genuine Article (R) Number: KC718
TI CHARACTERIZATION OF EHRLICHIAL ORGANISMS ISOLATED FROM A WILD MOUSE
AU KAWAHARA M; SUTO C; RIKIHISA Y (Reprint); YAMAMOTO S; TSUBOI Y
CS OHIO STATE UNIV, COLL VET MED, DEPT VET PATHOBIOL, COLUMBUS, OH, 43210; NAGOYA CITY PUBL HLTH RES INST, MIZUHO KU, NAGOYA 467, JAPAN; NAGOYA UNIV, SCH MED, DEPT MED ZOOL, NAGOYA, AICHI 466, JAPAN; MIYAZAKI PREFECTURE INST PUBL HLTH & ENVIRONM, MIYAZAKI 880, JAPAN; NATL INST HLTH, DEPT VET SCI, SHIMAGAWA KU, TOKYO 141, JAPAN
CYA USA; JAPAN
SO JOURNAL OF CLINICAL MICROBIOLOGY, (JAN 1993) Vol. 31, No. 1, pp. 89-96.
ISSN: 0095-1137.
DT Article; Journal
FS LIFE; CLIN
LA ENGLISH
REC Reference Count: 14
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 80 OF 85 VETU COPYRIGHT 2003 THOMSON DERWENT on STN
AB The etiology, clinical signs, pathogenesis, diagnosis and treatment of canine ehrlichiosis are reviewed. The main causative agent is E. **canis** while E. **chaffeensis**, E. ewingii, Cowdria ruminantium, E. risticii, E. equi and the agent of human granulocytic

ehrlichiosis can also cause infection. The IFAT is the most widely used diagnostic test for E. **canis** while **Western** blotting and PCR are also effective. Doxycycline and oxytetracycline are the treatments of choice for Ehrlichia infections. Imidocarb dipropionate is effective against E. **canis** but not E. risticii or E. **chaffeensis**. Enrofloxacin, penicillin, gentamycin, co-trimoxazole, chloramphenicol, pefloxacin and erythromycin are ineffective against E. **canis**. Supportive therapy includes fluids, blood transfusions, oxymetholone, nandrolone decanoate, iron, dexamethasone, prednisolone, vitamin B complex and levamisole.

AN 2000-62909 VETU
TI Canine ehrlichiosis: an update.
AU Kelly P J
LO Harare, Zimbabwe
SO J.S.Afr.Vet.Assoc. (71, No. 2, 77-86, 2000) 1 Tab. 133 Ref.
CODEN: JAVTAP
AV Biomedical Research and Training Institute, PO Box CY 1753, Causeway, Harare, Zimbabwe.
LA English
DT Journal
FA AB; LA; CT

L8 ANSWER 81 OF 85 VETU COPYRIGHT 2003 THOMSON DERWENT on STN
AB The first report of canine granulocytic ehrlichiosis (CGE) in 6 dogs from North Carolina and Virginia is presented. 5/6 Dogs presented with chronic nonregenerative anemia or polyarthritis while 1 was clinically normal. The IFAT and PCR amplification and sequencing were indicative of E. ewingii, E. equi and E. **canis** co-infection or cross-reactivity. All 6 dogs were given p.o. tetracycline or doxycycline, 3 blood transfusions, 4 p.o. prednisone and 4 p.o. phenylbutazone or aspirin. The response to treatment was variable, with those with polyarthritis responding the most quickly.

AN 1998-62253 VETU
TI Granulocytic ehrlichiosis in dogs from North Carolina and Virginia.
AU Goldman E E; Breitschwerdt E B; Grindem C B; Hegarty B C; Walls J J; Dumler J S
CS Univ.North-Carolina-State; Johns-Hopkins-Med.Inst.
LO Raleigh, N.C.; Ames, Iowa, USA
SO J.Vet.Intern.Med. (12, No. 2, 61-70, 1998) 3 Fig. 5 Tab. 48 Ref.
CODEN: JVIMEM
AV 4700 Hillsborough Street, Raleigh, NC 27606, U.S.A. (E.B.B.). (email: ebreitsc@snl.cvm.ncsu.edu).
LA English
DT Journal
FA AB; LA; CT

L8 ANSWER 82 OF 85 VETU COPYRIGHT 2003 THOMSON DERWENT on STN
AB A nested PCR method is described for the detection of Ehrlichia **canis** DNA in dogs. The **assay** was able to detect experimental infection before or at the time of seroconversion and was specific and sensitive. In blood samples from dogs from E. **canis** endemic and non-endemic areas, some from dogs previously treated with doxycycline (DO), the nested PCR compared favorably with immunofluorescence antibody **assay** (IFA). Thus the nested PCR may be more useful than IFA for assessing the clearance of organisms after antibiotic therapy, especially in areas where E. **canis** is endemic.

AN 1997-62110 VETU
TI Comparison of nested PCR with immunofluorescent antibody **assay** for detection of Ehrlichia **canis** infection in dogs treated with doxycycline.
AU Wen B; Rikihisa Y; Mott J M; Greene R; Kim H Y; Zhi N
CS Univ.Ohio-State
LO Columbus, Ohio; Phoenix, Ariz., USA

SO J.Clin.Microbiol. (35, No. 7, 1852-55, 1997) 5 Fig. 1 Tab. 21 Ref. CODEN: JCMIDW

AV Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 1925 Coffey Road, Columbus, OH 43210-1096, U.S.A. (Y.R., 9 authors).

LA English

DT Journal

FA AB; LA; CT

L8 ANSWER 83 OF 85 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2003) on STN

AB We report the first isolation and molecular and antigenic characterization of a human ehrlichial species in South America. A retrospective study was performed with serum specimens from 6 children with clinical signs suggestive of human ehrlichiosis and 43 apparently healthy adults who had a close contact with dogs exhibiting clinical signs compatible with canine ehrlichiosis. The evaluation was performed by the indirect fluorescent-antibody **assay** with Ehrlichia **chaffeensis** Arkansas, Ehrlichia **canis** Oklahoma, and Ehrlichia muris antigens. The sera from two apparently healthy humans were positive by the indirect fluorescent-antibody **assay** for all three antigens. Of the three antigens, samples from humans 1 and 2 showed the highest antibodies titers against E. **chaffeensis** and E. muris, respectively. The remaining serum samples were negative for all three antigens. One year later examination of a blood sample from subject 1 revealed morulae morphologically resembling either E. **canis**, E. **chaffeensis**, or E. muris in monocytes in the blood smear. The microorganism, referred to here as Venezuelan human ehrlichia (VHE), was isolated from the blood of this person at 4 days after coculturing isolated blood leukocytes with a dog macrophage cell line (DH82). The organism was also isolated from mice 10 days after intraperitoneal inoculation of blood leukocytes from subject 1. Analysis by electron microscopy showed that the human isolate was ultrastructurally similar to E. **canis**, E. **chaffeensis**, and E. muris. When the virulence of VHE in mice was compared with those of E. **chaffeensis** , B. **canis**, and E. muris, only VHE and E. muris induced clinical signs in BALB/c mice at 4 and 10 days, respectively, after intraperitoneal inoculation. VHE was reisolated from peritoneal exudate cells of the mice. Only E. **chaffeensis**- and E. muris-infected mice developed significant splenomegaly. **Western immunoblot** analysis showed that serum from subject 1 reacted with major proteins of the VHE antigen of 110, 80, 76, 58, 43, 35, and 34 kDa. Human serum against E. **chaffeensis** reacted strongly with 58-, 54-, 52-, and 40-kDa proteins of the VHE antigen. Anti-E. **canis** dog serum reacted strongly with 26- and 24-kDa proteins of VHE. In contrast, anti-E. sennetsu rabbit and anti-E. muris mouse sera did not react with the VHE antigen. Serum from subject 1 reacted with major proteins of 90, 64, or 47 kDa of the E. **chaffeensis**, B. **canis**, and E. muris antigens. This reaction pattern suggests that this serum sample was similar to serum samples from E. **chaffeensis**-infected human patients in Oklahoma. The base sequence of the 16S rRNA gene of VHE was most closely related to that of E. **canis** Oklahoma. On the basis of these observations, we suggest that VHE is a new strain or a subspecies of E. **canis** which may cause asymptomatic persistent infection in humans.

AN 97:62350 AGRICOLA

DN IND20588446

TI Ehrlichia **canis**-like agent isolated from a man in Venezuela: antigenic and genetic characterization.

AU Perez, M.; Rikihisa, Y.; Wen, B.

CS "Lisandro Alvarado" Centroccidental University, Barquisimeto-Lara State, Venezuela.

AV DNAL (QR46.J6)
SO Journal of clinical microbiology, Sept 1996. Vol. 34, No. 9. p. 2133-2139
Publisher: Washington : American Society for Microbiology,
CODEN: JCMIDW; ISSN: 0095-1137
NTE Includes references
CY District of Columbia; United States
DT Article
FS U.S. Imprints not USDA, Experiment or Extension
LA English

L8 ANSWER 84 OF 85 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2003) on STN
AB Currently available serological tests for cowdriosis (Cowdria ruminantium infection) in domestic ruminants are hampered by their low specificities because of cross-reactivity with Ehrlichia spp. The use of recombinant major antigenic protein (MAP1) of C. ruminantium for serodiagnosis was investigated. Overlapping fragments of the MAP1 protein were expressed in Escherichia coli and were reacted with sera from sheep infected with either C. ruminantium or Ehrlichia ovina. Two immunogenic regions on the MAP1 protein, designated MAP1-A and MAP1-B, were identified. MAP1-A was reactive with C. ruminantium antisera, E. ovina antisera, and three MAP1-specific monoclonal antibodies, whereas MAP1-B reacted only with C. ruminantium antisera. An indirect enzyme-linked immunosorbent **assay** (**ELISA**) based on MAP1-B was further developed and validated with sera from animals experimentally infected with C. ruminantium or several Ehrlichia spp. Antibodies raised in sheep, cattle, and goats against nine isolates of C. ruminantium reacted with MAP1-B. Cross-reactivity with MAP1-B was limited to Ehrlichia **canis** and Ehrlichia **chaffeensis**, two rickettsias which do not infect ruminants. Antibodies to Ehrlichia spp. which do infect ruminants (E. bovis, E. ovina, and E. phagocytophila) did not react with MAP1-B. Antibody titers to C. ruminantium in sera from experimentally infected cattle, goats, and sheep were detectable for 50 to 200 days postinfection. Further validation of the recombinant MAP1-B-based **ELISA** was done with sera obtained from sheep raised in heartwater-free areas in Zimbabwe and from several Caribbean islands. A total of 159 of 169 samples which were considered to be false positive by immunoblotting or indirect **ELISA** did not react with MAP1-B. In conclusion, recombinant MAP1-B may be a suitable antigen for a sensitive serological test for cowdriosis, with dramatically improved specificity.
AN 97:2524 AGRICOLA
DN IND20539504
TI Use of a specific immunogenic region on the Cowdria ruminantium MAP1 protein in a serological **assay**.
AU Vliet, A.H.M. van; Zeijst, B.A.M. van der.; Camus, E.; Mahan, S.M.; Martinez, D.; Jongejan, F.
CS Utrecht University, Utrecht, The Netherlands.
AV DNAL (QR46.J6)
SO Journal of clinical microbiology, Sept 1995. Vol. 33, No. 9. p. 2405-2410
Publisher: Washington : American Society for Microbiology,
CODEN: JCMIDW; ISSN: 0095-1137
NTE Includes references
CY District of Columbia; United States
DT Article
FS U.S. Imprints not USDA, Experiment or Extension
LA English

L8 ANSWER 85 OF 85 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2003) on STN
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genetically closely related, as determined by 16S rRNA gene base sequence comparison, but they exhibit biologic differences. E. **chaffeensis** is the etiologic agent of human ehrlichiosis. E. **canis** and E. ewingii cause two distinctly different forms of canine ehrlichiosis and infect different types of leukocytes, monocytes and granulocytes, respectively. E. **chaffeensis** can also infect dogs. In the study, **Western immunoblot** analysis of sera from dogs inoculated with E. **chaffeensis**, E. **canis**, or E. ewingii was performed to determine antigenic specificity and the intensities of the reactions to purified E. **chaffeensis** and E. **canis** antigens. At 2 to 3 weeks postexposure, antisera from four dogs inoculated with E. **chaffeensis** reacted with 64-, 47-, 31-, and 29-kDa proteins of E. **chaffeensis** but reacted poorly with E. **canis** antigen. In contrast, at 2 to 3 weeks postexposure, antisera from four E. **canis**-inoculated dogs reacted strongly with the 30-kDa major antigen of E. **canis** but reacted poorly with proteins from E. **chaffeensis**. At 4 weeks postexposures the sera from three E. ewingii-inoculated dogs showed weak binding to 64- and 47-kDa proteins of both E. **chaffeensis** and E. **canis**. Convalescent-phase sera from human ehrlichiosis patients and sera from dogs chronically infected with E. ewingii strongly reacted with similar sets of proteins of E. **chaffeensis** and E. **canis** with similar intensities. However, sera from dogs chronically infected with E. **canis** reacted more strongly with a greater number of E. **canis** proteins than with E. **chaffeensis** proteins. The protein specificity described in the report suggests that dogs with E. **canis** infections can be distinguished from E. **chaffeensis** -infected animals by **Western immunoblot** analysis with both E. **canis** and E. **chaffeensis** antigens.

AN 95:25855 AGRICOLA
DN IND20454807
TI **Western immunoblot** analysis of Ehrlichia **chaffeensis**, E. **canis**, or E. ewingii infections in dogs and humans.
AU Rikihisa, Y.; Ewing, S.A.; Fox, J.C.
CS Ohio State University, Columbus, OH
AV DNAL (QR46.J6)
SO Journal of clinical microbiology, Sept 1994. Vol. 32, No. 9. p. 2107-2112
Publisher: Washington : American Society for Microbiology,
CODEN: JCMIDW; ISSN: 0095-1137
NTE Includes references
CY District of Columbia; United States
DT Article
FS U.S. Imprints not USDA, Experiment or Extension
LA English

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